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=> d his

     (FILE 'HOME' ENTERED AT 07:48:12 ON 18 OCT 1999)

     FILE 'HCAPLUS' ENTERED AT 07:48:30 ON 18 OCT 1999
L1          1946 S ERBB2 OR ERBB 2 OR ERB B2
L2        127943 S ANTIBOD?
L3           245 S L1 (L) L2
L4        328668 S RECEPTOR#
L5           142 S L3 (L) L4
L6          3277 S INTERNALIZ?
L7             2 S L5 AND L6
L8             4 S L1 AND L2 AND L6
L9           232 S L1 AND L2 AND L4
L10           25 S L9 AND CHIMER?
L11         1001 S L1 (L) L4
L12           23 S L11 AND L2 AND  CHIMER?
L13          384 S CDR OR COMPLEMENTARITY DETERMIN? REGION?
L14            3 S L11 AND L13
L15           29 S L8 OR L12 OR L14

     FILE 'WPIDS' ENTERED AT 07:53:24 ON 18 OCT 1999
L16           78 S L1
L17           39 S L16 AND ANTIBOD?
L18            6 S L17 AND (INTERNALI? OR CHIMER?)
L19            4 S L17 AND (CDR OR COMPLEMENTARITY DETERMIN? REGION?)
L20           19 S L17 AND RECEPTOR#
L21            7 S L18 OR L19
L22           19 S L20 NOT L7
L23            0 S L20 NOT L17

     FILE 'BIOSIS' ENTERED AT 07:55:22 ON 18 OCT 1999
L24         3057 S L1
L25          620 S L24 AND ANTIBOD?
L26          312 S L25 AND RECEPTOR#
L27           27 S L26 AND (INTERNALI? OR CHIMER?)
L28            0 S L26 AND (CDR OR COMPLEMENTARITY DETERMIN? REGION?)

     FILE 'BIOSIS, WPIDS, HCAPLUS' ENTERED AT 07:56:34 ON 18 OCT 1999
L29           54 DUP REM L27 L21 L15 (9 DUPLICATES REMOVED)

=> d bib ab 1-54

L29  ANSWER 1 OF 54  WPIDS COPYRIGHT 1999   DERWENT INFORMATION LTD
AN   1999-152878 [13]   WPIDS
CR   1992-007427 [01];  1992-284329 [34];  1993-368719 [46];  1997-020415
[02]
DNC  C1999-045099
TI   Antibody specific for 30 kD erbB-2 ligand -
     useful for detecting ligand in cancer prognosis or diagnosis.
DC   B04 D16
IN   LIPPMAN, M E; LUPU, R
PA   (LIPP-I) LIPPMAN M E; (LUPU-I) LUPU R
CYC  1
PI   US 5869618    A  19990209 (199913)*      61p
ADT  US 5869618 A Cont of US 1990-528438 19900525, CIP of US 1991-640497
     19910114, CIP of US 1992-872114 19920422, CIP of US 1992-875788 19920429,
```

CIP of US 1992-917988 19920724, Div ex US 1993-96277 19930726, US 1995-550815 19951031
FDT US 5869618 A Div ex US 5578482
PRAI US 1993-96277 19930726; US 1990-528438 19900525; US 1991-640497 19910114; US 1992-872114 19920422; US 1992-875788 19920429; US 1992-917988 19920724; US 1995-550815 19951031
AB US 5869618 A UPAB: 19990813
An **antibody** that specifically binds a protein having the following characteristics is new: it binds to heparin-Sepharose; it has a molecular weight of about 30 kD by SDS-PAGE; it has a molecular weight of about 22 kD by SDS-PAGE after N-glycanase digestion; it has the amino acid sequence of a 22 kD polypeptide produced by in-vitro translation of polyA RNA from MDA-MB-231 cells; upon hydrolysis with S. aureus V8 protease or elastase, it produces the same peptide pattern as the 22 kD polypeptide produced by in-vitro translation of polyA RNA from MDA-MB-231 cells; it induces phosphorylation of **erbB-2** protein p185 in cells that overexpress **erbB-2**; it induces **internalisation** of the **erbB-2** receptor; it stimulates growth of **erbB-2**-overexpressing cells at low concentrations; it inhibits growth of **erbB-2** -overexpressing cells at high concentrations; it reverses Mab 4D5-dependent inhibition of **erbB-2**-overexpressing cells; it induces differentiation of **erbB-2** -overexpressing cells; and it stimulates phosphorylation of epidermal growth factor receptor (EGFR) in EGFR-expressing cells. The **antibody** does not cross-react with TGF alpha , EGF, amphiregulin or HB-EGF.
USE - The **antibody** can be used to detect the 30 kD **erbB-2** ligand [gp30] in serum or urine samples as a prognostic/diagnostic marker for tumour progression.
Dwg.0/25

L29 ANSWER 2 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:376422 BIOSIS
DN PREV199900376422
TI Relative cytotoxic activity of immunotoxins reactive with different epitopes on the extracellular domain of the c-**erbB-2** (HER-2/neu) gene product p185.
AU Boyer, Cinda M.; Pusztai, Lajos; Wiener, Jon R.; Xu, Feng Ji; Dean, G. Scott; Bast, Blanche S.; O'Briant, Kathy C.; Greenwald, Marilee; DeSombre, Karen A.; Bast, Robert C., Jr. (1)
CS (1) Division of Medicine, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX, 77030 USA
SO International Journal of Cancer, (Aug. 12, 1999) Vol. 82, No. 4, pp. 525-531.
ISSN: 0020-7136.
DT Article
LA English
SL English
AB Different epitopes on the extracellular domain of the HER-2 **receptor** can serve as distinct targets for immunotoxins. To determine the optimal epitope target for immunotoxin therapy, 7 anti-HER-2 ricin A chain murine monoclonal immunotoxins, each reactive with different

epitopes of HER-2 **receptor**, were tested for cytotoxic activity. The immunotoxins produced 1.2-4.6 logs of cytotoxicity in limiting dilution clonogenic assays with 2 breast cancer cell lines that overexpressed HER-2. Cytotoxicity did not correlate with immunoglobulin isotype, binding affinity, relative position of epitopes or **internalization** of the anti-HER-2 immunotoxins. Interestingly, the most and least effective immunotoxins bound to epitopes in very close proximity. Competitive binding assays with unconjugated **antibodies** have previously indicated that our **antibodies** recognized epitopes that are arranged in a linear array. To orient this relative epitope map, deletions were prepared in the HER-2/neu gene and these mutant constructs were expressed in NIH3T3 cells. Epitope expression was determined by **antibody** binding and radioimmunoassay. Epitopes targeted by the PB3, 454C11 and NB3 **antibodies** are localized N-terminal to the epitopes recognized by ID5, BD5, 741F8 and 520C9 **antibodies**. The 2 non-conformational epitopes PB3 and NB3 were localized to regions corresponding to amino acides 78-242 of the p185HER-2 protein.

L29 ANSWER 3 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 1
AN 1999:145729 BIOSIS
DN PREV199900145729
TI Toward selection of **internalizing antibodies** from phage libraries.
AU Becerril, Baltazar; Poul, Marie-Alix; Marks, James D. (1)
CS (1) Dep. Anesthesia, Univ. California, San Francisco, Room 3C-38, San Francisco General Hosp., 1001 Potrero Ave., San Francisco, CA 94110 USA
SO Biochemical and Biophysical Research Communications, (Feb. 16, 1999) Vol. 255, No. 2, pp. 386-393.
ISSN: 0006-291X.
DT Article
LA English
AB **Antibodies** which bind cell surface **receptors** in a manner whereby they are endocytosed are useful molecules for the delivery of drugs, toxins, or DNA into the cytosol of mammalian cells for therapeutic applications. Traditionally, **internalizing antibodies** have been identified by screening hybridomas. For this work, we studied a human scFv (C6.5) which binds **ErbB2** to determine the feasibility of directly selecting **internalizing antibodies** from phage libraries and to identify the most efficient display format. Using wild-type C6.5 scfv displayed monovalently on a phagemid, we demonstrate that anti-**ErbB2** phage **antibodies** can undergo **receptor**-mediated endocytosis. Using affinity mutants and dimeric diabodies of C6.5 displayed as either single copies on a phagemid or multiple copies on phage, we define the role of affinity, valency, and display format on phage endocytosis and identify the factors that lead to the greatest enrichment for **internalization**. Phage displaying bivalent diabodies or multiple copies of scfv were more efficiently endocytosed than phage displaying monomeric scfv and recovery of infectious phage was increased by preincubation of cells with chloroquine. Measurement of phage recovery from within the cytosol as a function of applied phage titer indicates that it is possible to select for endocytosable **antibodies**, even at the low concentrations that would exist for a single phage **antibody** member in a library of 109.

L29 ANSWER 4 OF 54 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1998-322712 [28] WPIDS
DNC C1998-099337
TI New **chimeric** enzymes, particularly for detection of analytes - comprising a starting enzyme in which a mimetope is inserted to bind a binding molecule to modulate the activity of the enzyme.
DC B04 C07 D16 J04
IN FASTREZ, J; LEGENDRE, D; SOUMILLION, P
PA (UYLO-N) UNIV CATHOLIQUE LOUVAIN
CYC 77
PI WO 9823731 A2 19980604 (199828)* EN 60p
RW: AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW
AU 9855719 A 19980622 (199844)
ADT WO 9823731 A2 WO 1997-IB1643 19971126; AU 9855719 A AU 1998-55719 19971126
FDT AU 9855719 A Based on WO 9823731
PRAI US 1996-757425 19961127
AB WO 9823731 A UPAB: 19980715
A **chimeric** enzyme (CE) comprises: (a) a starting enzyme which is a polypeptide; and (b) a mimotope comprising at least one amino acid, which mimotope is inserted into the starting enzyme or which replaces at least one amino acid of the starting enzyme, the insertion or replacement yielding the CE which has an enzymatic activity of the starting enzyme and
the activity of the CE is modulated upon binding of a binding molecule (BM) to the mimotope. Also claimed is a method for detecting or quantifying an anaylate in a test sample comprising contacting the CE with
a test sample, a BM which binds to the mimotope of the CE and a substrate for the CE, detecting the amount of catalysis of the substrate where the BM or the anaylate modulates this catalysis.
USE - The BM can bind to the CEs to modulate the catalysis of a substrate by the CEs. The CEs can be used for the detection of BMs which can be analytes such as an **antibody** specific for prostate specific antigen (PSA), carcinoma embryonic antigen (CEA), c-**erbB2**, products of oncogenes, virus (e.g. HIV or hepatitis), or bacteria (e.g. Staphylococcus). Alternatively the CEs can be used for the detection of ligands of the BMs.
Dwg.0/6

L29 ANSWER 5 OF 54 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1998-311318 [27] WPIDS
CR 1993-272889 [34]; 1996-333194 [33]; 1999-023541 [02]
DNC C1998-095897
TI Imaging of antigens in vivo - using dimers of single-chain **antibody** Fv fragments.
DC B04 D16 K08
IN HOUSTON, L L; HUSTON, J S; OPPERMANN, H; RING, D B
PA (CHIR) CHIRON CORP; (CREA-N) CREATIVE BIOMOLECULES INC
CYC 1
PI US 5753204 A 19980519 (199827)* 30p
ADT US 5753204 A CIP of US 1992-831967 19920206, Div ex US 1993-133804

19931007, US 1995-461838 19950605
FDT US 5753204 A Div ex US 5534254
PRAI US 1993-133804 19931007; US 1992-831967 19920206; US 1995-461838 19950605
AB US 5753204 A UPAB: 19990113
A method (A) of imaging a preselected antigen expressed in a mammal, comprises: (a) administering to the mammal at a concentration sufficient for extracorporeal detection of the preselected antigen, a composition comprising a pharmaceutically acceptable carrier in combination with a dimeric biosynthetic construct that binds at least one preselected antigen, where the construct comprises two separate polypeptide chains, each of which has an amino acid sequence defining a single chain Fv (sFv) comprising two polypeptide domains connected by a polypeptide linker spanning the distance between the C terminus of one domain and the N terminus of the other, the amino acid sequence of each domain comprising **complementarity determining regions** (CDRs) interposed between framework regions (FRs), the CDRs and FRs of each sFv together defining a binding site immunologically reactive with a preselected antigen, and a C terminal tail essentially free of helical character under physiological conditions and comprising at least one amino acid having a derivatisable amino acid side chain, and an sFv coupler linking together each sFv through the derivatisable amino acid side chain disposed within the C terminal tail of each sFv, the dimeric biosynthetic construct having a conformation where the binding site of each sFv is operative to bind a preselected antigen when the dimeric biosynthetic construct is administered to the mammal; and (b) detecting the dimeric biosynthetic construct bound to a preselected antigen. Also claimed is a method (B) of imaging a preselected antigen expressed in a mammal, comprising: (a) administering to the mammal at a concentration sufficient for extracorporeal detection of a preselected antigen a composition comprising a pharmaceutically acceptable carrier in combination with a dimeric biosynthetic construct that binds at least one preselected antigen, where the construct comprises two separate polypeptide chains, each of which has an amino acid sequence defining an sFv comprising two polypeptide domains connected by a polypeptide linker spanning the distance between the C terminus of one domain and the N terminus of the other, the amino acid sequence of each domain comprising CDRs interposed between FRs, the CDRs and FRs of each sFv together defining a binding site immunologically reactive with a preselected antigen, and an N terminal tail comprising at least one amino acid having a derivatisable amino acid side chain, and an sFv coupler linking together each sFv through the derivatisable amino acid side chain disposed within the N terminal tail of each sFv, the dimeric biosynthetic construct having a conformation where the binding site of each sFv is operative to bind a preselected antigen when the dimeric biosynthetic construct is administered to the mammal; and (b) detecting the dimeric biosynthetic construct bound to a preselected antigen. Also claimed is a method (C) of imaging a preselected antigen expressed in a mammal, comprising: (a) administering to the mammal at a concentration sufficient for extracorporeal detection of a preselected antigen, a composition comprising a pharmaceutically acceptable carrier in combination with a dimeric biosynthetic construct that binds at least one preselected antigen, where the construct comprises two separate

polypeptide chains, which have an amino acid sequence defining an sFv comprising two polypeptide domains connected by a polypeptide linker spanning the distance between the C terminus of one domain and the N terminus of the other, the amino acid sequence of each domain comprising CDRs interposed between FRs, the CDRs and FRs of each sFv together defining a binding site immunologically reactive with a preselected antigen, one of the polypeptide chains further comprising an amino acid sequence defining an N terminal tail comprising at least one amino acid having a derivatisable amino acid side chain, and the other of the polypeptide chains further comprising an amino acid sequence defining a C terminal tail comprising at least one amino acid having a derivatisable amino acid side chain; an sFv coupler linking together each sFv through the derivatisable amino acid side chain disposed within the N terminal tail of one polypeptide chain and the derivatisable amino acid side chain disposed within the C terminal tail of the other polypeptide chain, the dimeric biosynthetic construct having a conformation where the binding site of each sFv is operative to bind a preselected antigen when the dimeric biosynthetic construct is administered to the mammal; and (b) detecting the dimeric biosynthetic construct bound to a preselected antigen.

USE - The method is for use in magnetic resonance imaging of c-**erbB-2** or related antigens in cancer diagnosis.

ADVANTAGE - The biosynthetic constructs have enhanced properties as I(in vivo)I targetting agents in comparison with intact monoclonal **antibodies** or their Fab fragments. The dimeric constructs permit the I(in vivo)I targetting of an epitope on an antigen with greater apparent avidity, including greater tumour specificity, tumour localisation and tumour retention properties than that of the Fab fragment having the same CDRS as the construct.

Dwg.0/6

L29 ANSWER 6 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1998:618835 HCAPLUS
DN 129:229686
TI Costimulation of T-cell proliferation by a **chimeric** bispecific costimulatory protein
IN Wels, Winfried; Gerstmayer, Bernhard
PA Boehringer Mannheim Gmbh, Germany
SO PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9839033	A1	19980911	WO 1998-EP1009	19980221
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9868222	A1	19980922	AU 1998-68222	19980221
PRAI	EP 1997-103497		19970304		

WO 1998-EP1009 19980221

AB A sol. bispecific fusion protein consisting of: a) a binding domain which recognizes a sp. surface mol. on a target cell, covalently linked to b) a domain capable of stimulating T-cell proliferation, can be used for a specific costimulation of a T-cell directed against said target cell. Thus, chimeric gene encoding the extracellular domain of human B7-2 fused to the ErbB2-specific scFv(FRP5) antibody domain was construct, expressed, and test for binding to CD28 and CTLA-4-expressing T cell and providing costimulation for the proliferation of syngeneic T cells.

L29 ANSWER 7 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS

AN 1998:430305 BIOSIS

DN PREV199800430305

TI ErbB-1 and **ErbB-2** acquire distinct signaling properties dependent upon their dimerization partner.

AU Olayioye, Monilola A.; Graus-Porta, Dianha; Beerli, Roger R.; Rohrer, Jack; Gay, Brigitte; Hynes, Nancy E. (1)

CS (1) Friedrich Miescher Inst., PO Box 2543, CH-4002 Basel Switzerland

SO Molecular and Cellular Biology, (Sept., 1998) Vol. 18, No. 9, pp. 5042-5051.
ISSN: 0270-7306.

DT Article

LA English

AB The different epidermal growth factor (EGF)-related peptides elicit a diverse array of biological responses as the result of their ability to activate distinct subsets of ErbB **receptor** dimers, leading to the recruitment of different intracellular signaling networks. To specifically examine dimerization-dependent modulation of **receptor** signaling, we constructed NIH 3T3 cell lines expressing ErbB-1 and **ErbB-2** singly and in pairwise combinations with each other ErbB family member. This model system allowed the comparison of EGF-activated ErbB-1 with ErbB-1 activated by Neu differentiation factor (NDF)-induced heterodimerization with ErbB-4. In both cases, ErbB-1 coupled to the adaptor protein Shc, but only when activated by EGF was it able to interact with Grb2. Compared to the rapid **internalization** of EGF-activated ErbB-1, NDF-activated ErbB-1 showed delayed **internalization** characteristics. Furthermore, the p85 subunit of phosphatidylinositol kinase (PI3-K) associated with EGF-activated ErbB-1 in a biphasic manner, whereas association with ErbB-1 transactivated by ErbB4 was monophasic. The signaling properties of **ErbB-2** following heterodimerization with the other ErbB **receptors** or homodimerization induced by point mutation or monoclonal **antibody** treatment were also analyzed. **ErbB-2** binding to peptides containing the Src homology 2 domain of Grb2 or p85 and the phosphotyrosine binding domain of Shc varied according to the mode of **receptor** activation. Finally, EGF-activated ErbB-1 in a biphasic manner, whereas association with ErbB-1 transactivated by ErbB-4 was monophasic. The signaling properties of **ErbB-2** following heterodimerization with the other ErbB **receptors** or homodimerization induced by point mutation or monoclonal **antibody** treatment were also analyzed. **ErbB-2** binding to peptides containing the Src homology 2 domain of Grb2 or p85 and the phosphotyrosine binding domain of Shc varied according to the mode of **receptor** activation. Finally,

L29 ANSWER 8 OF 54 HCAPLUS COPYRIGHT 1999 ACS

AN 1999:7134 HCAPLUS
DN 130:221843
TI Production and characterization of a recombinant single-chain anti ErbB2-clavin immunotoxin
AU D'Alatri, Laura; Di Massimo, Anna Maria; Anastasi, Anna Maria; Pacilli, Aurelio; Novelli, Sabrina; Saccinto, Maria Pia; De Santis, Rita; Mele, Antonio; Parente, Dino
CS Menarini Ricerche S.p.A., Department of Biotechnology, Rome, 00040, Italy
SO Anticancer Res. (1998), 18(5A), 3369-3373
CODEN: ANTRD4; ISSN: 0250-7005
PB Anticancer Research
DT Journal
LA English
AB We generated a recombinant immunotoxin, named scFv(MGR6)-Cla, composed of the Fv region of an anti ErbB2 monoclonal antibody (MGR6) fused to clavin, a type 1 ribosome-inactivating protein (RIP) from Aspergillus clavatus. ErbB2 is a tyrosine kinase receptor which is overexpressed in most adenocarcinomas; clavin is a 17 kDa RNase which inhibits protein synthesis by inactivating ribosomes. A recombinant DNA construct contg. the cDNA of the single chain Fv fragment (scFv) of the MGR6 antibody fused to the clavin cDNA, was expressed at high levels in Escherichia coli as an insol. fusion protein contg. an N-terminal affinity tag of six consecutive histidine residues. Inclusion bodies were denatured and the recombinant fusion protein was purified under denaturing conditions by single-step purifn. using immobilized metal ion affinity chromatog. (IMAC). The purified immunotoxin was renatured at high yield and histidine tag removed by digestion with enterokinase. The purity of the immunotoxin obtained after refolding was confirmed by SDS-PAGE, RP-HPLC, GPC-HPLC and N-terminal sequence anal. Cell-free protein synthesis inhibition and binding assays showed that both clavin and scFv(MGR6) maintained their properties after refolding.

L29 ANSWER 9 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:207971 BIOSIS
DN PREV199800207971
TI The oncogenic **ErbB-2**/ErbB-3 heterodimer is a surrogate **receptor** of the epidermal growth factor and betacellulin.
AU Pinkas-Kramarski, Ronit; Lenferink, Anne E. G.; Bacus, Sarah S.; Lyass, Ljuba; Van De Poll, Monique L. M.; Klapper, Leah N.; Tzahar, Eldad; Sela, Michael; Van Zoelen, Everardus J. J.; Yarden, Yosef (1)
CS (1) Dep. Mol. Cell Biol., Weizmann Inst. Sci., Rehovot 76100 Israel
SO Oncogene, (March 12, 1998) Vol. 16, No. 10, pp. 1249-1258.
ISSN: 0950-9232.
DT Article
LA English
AB The ErbB-1 **receptor** tyrosine kinase binds to six different growth factors, whose prototype is the epidermal growth factor (EGF). Two homologous epithelial **receptors**, ErbB-3 and ErbB-4, bind all isoforms of another family of growth factors, the, Neu differentiation factors (NDFs/neuregulins). The fourth member of the ErbB family, **ErbB-2**, acts as the preferred heterodimeric partner of ligand-occupied complexes of the three other ErbB proteins. Here we report

that at high concentrations, EGF can induce cell growth and differentiation in the absence of ErbB-1. This function is shared by betacellulin, but not by three other ligands, including the transforming growth factor a (TGFalpha). The functional **receptor** was identified as a heterodimer between ErbB-3 and **ErbB-2**, a previously identified oncogenic complex. When singly expressed, neither ErbB-3 nor **ErbB-2** can mediate signaling by EGF. In addition, when co-expressed, blocking either **receptor** by using site-specific **antibodies** inhibited EGF and betacellulin activities, indicating strict cooperativity between ErbB-3 and **ErbB-2**. Through analysis of **chimeras** between EGF and TGFalpha, we identified the middle portion of EGF (loop B) as the site that enables activation of **ErbB-2**/ErbB-3. In conclusion, cooperative and promiscuous binding of stroma-derived growth factors by the epithelium-expressed **ErbB-2**/ErbB-3 heterodimer may be significant to cancer development. The mechanistic implications of our results for a model that attributes **receptor** dimerization to ligand bivalency, as well as to a recently proposed mechanism of secondary dimerization, are discussed.

L29 ANSWER 10 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 2
AN 1998:396143 BIOSIS
DN PREV199800396143
TI Human tumor growth suppression by apoptosis induced with anti-**ErbB -2 chimeric** monoclonal **antibody**.
AU Sasaki, Shigeru (1); Tsujisaki, Masayuki; Jinnohara, Tsuneharu; Ishida, Tdao; Sekiya, Masuo; Adachi, Masaaki; Takahashi, Shuji; Hinoda, Yuji; Imai, Kohzoh
CS (1) First Dep. Intern. Med., Sapporo Med. Univ., S-1 W-16, Chuo-ku, Sapporo 060 Japan
SO Japanese Journal of Cancer Research, (May, 1998) Vol. 89, No. 5, pp. 562-570.
ISSN: 0910-5050.
DT Article
LA English
AB We established an anti-**ErbB-2** mouse-human **chimeric** monoclonal **antibody** (MoAb), CH401, which was able to kill cancer cells overexpressing the **ErbB-2** protein in vitro. The analysis of the killing mechanism indicated that MoAb CH401 might be the first anti-**ErbB-2** mouse-human **chimeric** MoAb which can induce the apoptosis of cancer cells, since morphological changes and DNA fragmentation were recognized in MoAb CH401-treated cells. The **ErbB-2 receptor** appears to have two opposing functions: acting as a **receptor** both for a growth factor and for an apoptotic factor. Our results indicate that MoAb CH401 treatment may prove to be very useful for cancer therapy.

L29 ANSWER 11 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:306103 BIOSIS
DN PREV199800306103
TI The level of **erbB2** expression predicts sensitivity to the cytotoxic effects of an intracellular anti-**erbB2** sFv.
AU Grim, Jon; Deshane, Jessy; Siegal, Gene P.; Alvarez, Ronald D.; Difiore, Paolo; Curiel, David T. (1)
CS (1) Dep. Pathol., Univ. Ala., Birmingham, AL 35294 USA
SO Journal of Molecular Medicine (Berlin), (May, 1998) Vol. 76, No. 6, pp.

451-458.
ISSN: 0946-2716.
DT General Review
LA English
AB We have previously demonstrated that an intracellular **antibody** (sFv) directed against **erbB2** can achieve a specific cytotoxicity in **erbB2** overexpressing cancer cells of varying histogenesis. In order to further delineate the mechanistic basis of the induced apoptosis, transient and stable cotransfections were performed. Transient cotransfection of **erbB2** mutant and **chimeric** molecules demonstrated that the cytoplasmic domain of **erbB2**, or the homologous cytoplasmic domain of the epidermal growth factor **receptor**, is required for apoptosis induction. These results were confirmed in assays utilizing differential derivation of stable clones. To examine the effects of varying ratios of the anti-**erbB2** sFv and its target **erbB2** we performed additional cotransfection experiments in **erbB2** negative target cells. When **erbB2** levels are held constant, observed cytotoxicity is proportional to the amount of sFv added. In addition, when sFv levels are held constant, increasing levels of cotransfected **erbB2** can overcome the apoptotic response. These results indicate that a minimal threshold level of the sFv and its target are required to induce cytotoxicity. To examine this phenomenon in an **erbB2** positive cell line, SKOV3 ovarian carcinoma cells were utilized to derive a stable clone expressing low levels of sFv. When this cell line was compared to the parental SKOV3 cell line, it was shown that less exogenous sFv was needed to induce cytotoxicity in the clone already expressing low levels of sFv, indicating that endogenous and exogenous levels of sFv are additive. In summary, the results presented here indicate that the carboxy-terminus of the intracellular domain of the **erbB2** molecule is involved in the induction of apoptosis. Furthermore, the expression levels of the sFv and its target protein need to overcome a threshold level in order to achieve a cytotoxic response.

L29 ANSWER 12 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:459213 BIOSIS
DN PREV199800459213
TI Targeted delivery of effector functions by **chimeric** **antibody**-Pseudomonas exotoxin A fusion proteins.
AU Fominaya, J.; Schmidt, M.; Uherek, C.; Wels, W.
CS Inst. Exp. Cancer Res., Tumor Biol. Cent., Breisacher Str. 117, D-79106 Freiburg Germany
SO Zentralblatt fuer Bakteriologie Supplement, (1998) Vol. 29, pp. 443-450.
Meeting Info.: Eighth European Workshop on Bacterial Protein Toxins Staffelstein, Kloster Banz, Germany June 29-July 4, 1997
ISSN: 0941-018X.
DT Conference
LA English

L29 ANSWER 13 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:358747 BIOSIS
DN PREV199800358747
TI Eps8, a tyrosine kinase substrate, is recruited to the cell cortex and dynamic F-actin upon cytoskeleton remodeling.

AU Provenzano, Claudia; Gallo, Rita; Carbone, Roberta; Di Fiore, Pier Paolo; Falcone, Germana; Castellani, Loriana; Alema, Stefano (1)
CS (1) Ist. Biol. Cell., CNR, Viale Marx 43, 00137 Rome Italy
SO Experimental Cell Research, (July 10, 1998) Vol. 242, No. 1, pp. 186-200.
ISSN: 0014-4827.
DT Article
LA English
AB Eps8 is a recently identified substrate of **receptor** and nonreceptor tyrosine kinases implicated in the control of cell proliferation. To investigate potential functions of Eps8, its intracellular localization has been examined in several cell types. In cycling fibroblasts immunolabeling with **antibodies** to Eps8 reveals a punctate pattern within the perinuclear region and staining of motile peripheral cell extensions and cell-cell contact regions. Stimulation of quiescent Swiss 3T3 fibroblasts with serum induces a striking reorganization of the actin cytoskeleton which is accompanied by the enrichment of Eps8 and cortactin in membrane ruffles and lamellipodia.
A similar accumulation of Eps8 to membrane ruffles is observed in cells treated with phorbol esters, which also induce marked changes of the F-actin cytoskeleton. The localization of Eps8 at the cell cortex is largely independent from the binding of Eps8 to an EGFR/**ErbB-2 chimeric receptor**. Moreover, fractionation studies reveal that a portion of the Eps8 molecules present in the cell periphery, unlike cortactin and the **receptor**, is resistant to mild extraction with detergent. Upon cellular transformation by the tyrosine kinase v-Src, a pool of Eps8 is recruited to newly formed specialized regions of the cytoskeleton, such as actin bodies in terminally differentiated myotubes and podosomes in fibroblasts, where cortactin and a variety of cytoskeletal proteins are also found. Extraction with Triton X-100 preserves the association of Eps8 to podosomes and leaves the majority of the v-Src tyrosine-phosphorylated Eps8 in the detergent-resistant fraction. The observed recruitment of Eps8 to highly dynamic cytoskeletal structures of normal and transformed cells suggests that Eps8 may play a role in the reorganization of the cytoskeleton, perhaps acting as a docking site for other signaling molecules.

L29 ANSWER 14 OF 54 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD DUPLICATE 3
AN 1997-077488 [07] WPIDS
DNN N1997-064299 DNC C1997-024928
TI New C6 human **antibody** binding specifically to c-**erbB-2** - useful for treatment and diagnosis of tumours, with reduced risk of generating immune response.
DC B04 D16 S03
IN MARKS, J D; SCHIER, R
PA (REGC) UNIV CALIFORNIA
CYC 22
PI WO 9700271 A1 19970103 (199707)* EN 117p
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP MX
AU 9661133 A 19970115 (199718)
EP 873363 A1 19981028 (199847) EN

R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
ADT WO 9700271 A1 WO 1996-US10287 19960613; AU 9661133 A AU 1996-61133 19960613; EP 873363 A1 EP 1996-918487 19960613, WO 1996-US10287 19960613
FDT AU 9661133 A Based on WO 9700271; EP 873363 A1 Based on WO 9700271
PRAI US 1995-250 19950615; US 1995-238 19950614
AB WO 9700271 A UPAB: 19970212
A C6 human **antibody** (Ab) that binds specifically to c-**erbB-2** is new. Also new are: (1) nucleic acid (I) encoding Ab; (2) cell contg. recombinant (I); (3) **chimeric** molecule (II) that binds specifically to tumour cells carrying c-**erbB-2**, consisting of an effector cpd. attached to the Ab; (4) polypeptide (A) contg. 1 or more **complementarity determining regions** (**CDR**) having amino acid sequences tabulated in the specification; (5) nucleic acid (Ia) encoding a single chain polypeptide (B) with the binding specificity of Ab and comprising the binding portions of variable regions of light and heavy chains of Ab, joined by a linker; (6) (B); and (7) expression cassettes contg. (Ia) and control sequences.

USE - Where the effector cpd. is a cytotoxin, (II) are used to inhibit growth of c-**erbB-2** positive tumours (esp. breast and other carcinomas) and where it is a label they are used to detect such cells, including in vivo localisation (claimed). Ab can also be used for diagnosis/localisation, in vivo or in vitro, esp. by immunoassay. (I) and (Ia) are used to produce recombinant proteins by standard methods (claimed).

ADVANTAGE - Unlike known anti-c-**erbB-2** **antibodies**, Ab are fully human, so should elicit little, if any, immunogenic response.

Dwg.0/4

L29 ANSWER 15 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1997:315481 HCAPLUS
DN 127:32753
TI Costimulation of T cell proliferation by a **chimeric** B7-2 **antibody** fusion protein specifically targeted to cells expressing the erbB2 proto-oncogene
AU Gerstmayer, Bernhard; Altenschmidt, Uwe; Hoffmann, Michael; Wels, Winfried
CS Inst. Experimental Cancer Research, Tumor Biology Center, Freiburg, D-79106, Germany
SO J. Immunol. (1997), 158(10), 4584-4590
CODEN: JOIMA3; ISSN: 0022-1767
PB American Association of Immunologists
DT Journal
LA English
AB T cells require at least 2 signals for activation and clonal expansion. The first signal conferring specificity is initiated by interaction of the T cell receptor with antigenic peptides in the context of MHC mols. The second, costimulatory signal can be provided by cell surface mols. on APCs such as B7-1 (CD8) and B7-2 (CD86), which interact with their counter-receptors on T cells. The absence of costimulatory signals presents one possible mechanism for tumor cells to escape immune surveillance. In exptl. models transfection of B7 genes into tumor cells can result in T cell-dependent tumor rejection. The authors developed a

novel approach to direct the costimulatory B7-2 mol. to the surface of target cells. Their approach is based on a chimeric fusion protein that consists of the extracellular domain of human B7-2 fused to a single-chain Ab domain (scFv) specific for the ErbB2 protein, a type I growth factor receptor overexpressed in a high percentage of human adenocarconimas. This B7-2225-scFv(FRP5) mol. expressed in the yeast Pichia pastoris and purified from culture supernatants is functionally active and binds to B7 counter-receptors and to ErbB2. B7-2225-scFv(FRP5) localizes specifically to the surface of ErbB2-expressing target cells, thereby providing a costimulatory signal that results in enhanced proliferation of syngeneic T cells. Effective tumor vaccines for cancer immunotherapy thus could be created by targeting such chimeric ligands to the surface of tumor cells.

L29 ANSWER 16 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:80476 BIOSIS
DN PREV199800080476
TI Costimulation of T-cell proliferation by a **chimeric** B7-**antibody** fusion protein.
AU Gerstmayer, Bernhard; Hoffmann, Michael; Altenschmidt, Uwe; Wels, Winfried (1)
CS (1) Inst. Exp. Cancer Res., Tumor Biol. Cent., Breisacher Str. 117, D-79106 Freiburg Germany
SO Cancer Immunology Immunotherapy, (Nov.-Dec., 1997) Vol. 45, No. 3-4, pp. 156-158.
ISSN: 0340-7004.
DT Article
LA English
AB T cells require at least two signals for activation and clonal expansion. The first signal conferring specificity is initiated by interaction of the T cell **receptor** with peptide-bearing MHC molecules. The second, costimulatory signal can be provided by cell-surface molecules on antigen-presenting cells such as B7-1 (CD80) and B7-2 (CD86), which interact with CD28 on T cells. To direct the costimulatory B7-2 molecule to the surface of tumor cells we have constructed a **chimeric** fusion protein, which consists of the extracellular domain of human B7-2 fused to a single-chain **antibody** domain (scFv) specific for the **ErbB2** protein, a type I growth factor **receptor** overexpressed in a high percentage of human adenocarcinomas. This B7-2225-scFv(FRP5) molecule, expressed in the yeast Pichia pastoris and purified from culture supernatants, binds to B7 counter-**receptors** and to **ErbB2**. B72225-scFv(FRP5) localizes specifically to the surface of **ErbB2**-expressing target cells, thereby providing a costimulatory signal, which results in enhanced proliferation of syngeneic T cells.

L29 ANSWER 17 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1997:132790 HCAPLUS
DN 126:143315
TI Therapeutic compounds comprised of anti-Fc receptor **antibodies**
IN Deo, Yashwant M.; Goldstein, Joel; Graziano, Robert; Somasundaram, Chezian

PA Medarex, Inc., USA
SO PCT Int. Appl., 113 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9640789	A1	19961219	WO 1996-US9988	19960607
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN				
CA 2220461	AA	19961219	CA 1996-2220461	19960607
AU 9663835	A1	19961230	AU 1996-63835	19960607
EP 832135	A1	19980401	EP 1996-923279	19960607
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
CN 1203603	A	19981230	CN 1996-196166	19960607
JP 11501522	T2	19990209	JP 1996-502133	19960607

PRAI US 1995-484172 19950607
WO 1996-US9988 19960607

AB Disclosed are multispecific multivalent mols. comprising an anti-FcR portion, an anti-target portion, and optionally an anti-enhancement factor portion. The multispecific antibodies are useful for treating cancer or infectious diseases. In example, bispecific antibodies of FcR-anti-her2/neu, H22 hinge region-epidermal growth factor, H2-heregulin (or gp30), H22-bombesin, H22-tetanus toxin, and anti-Fc.gamma.RI-anti-carcinoembryonic antigen were prepd. The tumor cell killing activity, T cell proliferation stimulating activity, or antigen-presenting activity of the resp. chimeric antibodies were tested.

L29 ANSWER 18 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1996:590542 HCAPLUS
DN 125:214272
TI Gene therapy using **chimeric** gene encoding receptors comprising inducer-responsive domains as well as proliferation or other signaling domains
IN Capon, Daniel J.; Smith, Douglas H.; Tian, Huan; Winslow, Genine A.; Siekevitz, Miriam
PA Cell Genesys, Inc., USA
SO PCT Int. Appl., 117 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9624671	A1	19960815	WO 1996-US1600	19960206
W: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, AZ, BY, KG, KZ				

RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

CA 2221571	AA	19960815	CA 1996-2221571	19960206
AU 9647761	A1	19960827	AU 1996-47761	19960206
EP 871726	A1	19981021	EP 1996-903786	19960206

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE

PRAI US 1995-384033 19950206
WO 1996-US1600 19960206

AB Multispecific chimeric receptor DNA sequences, expression cassettes and vectors contg. these sequences as well as cells contg. the chimeric DNA and chimeric receptor proteins expressed from the sequences are provided in the present invention. The multispecific chimeric receptor DNA and amino acid sequences comprise at least three domains that do not naturally exist together: (1) a multispecific binding domain comprising at least two extracellular inducer-responsive clustering domains which serves to bind at least one specific inducer mol.; (2) a transmembrane domain; and (3) a cytoplasmic domain which contains either a proliferation signaling domain that signals cells to divide, and effector function signaling domain which directs a host cell to perform its specialized function, or both an effector function signaling domain and a proliferation signaling domain. Optionally, the multispecific chimeric receptors may contain one or more intracellular inducer-responsive clustering domains attached to one or more cytoplasmic signaling domains or the transmembrane domain. The present invention also includes methods of using cells expressing these chimeric receptors for treatment of cancer, and autoimmune and infectious diseases.

L29 ANSWER 19 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1996:354001 HCAPLUS
DN 125:31930
TI **CDR**-grafted **antibodies** with heavy chain variable regions incorporated into light chain frameworks
IN Ill, Charles R.; Ludwig, James Richard; Rathnachalam, Radhakrishnan
PA Lilly, Eli, and Co., USA
SO PCT Int. Appl., 163 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9606625	A1	19960307	WO 1995-US10791	19950825

W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM

RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9534153	A1	19960322	AU 1995-34153	19950825

PRAI US 1994-296625 19940826
WO 1995-US10791 19950825

AB CDR-grafted antibodies in which donated heavy-chain CDRs are incorporated into the variable domain are grafted into the framework regions of the light chain of the acceptor antibody are described. To increase the binding of the CDRs and the secretion of multi-chain constructs, the antibodies are altered using techniques of mol. modeling to design the necessary changes. Modeling and construction of such chimeric antibodies is demonstrated.

L29 ANSWER 20 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1997:15509 HCAPLUS
DN 126:70662
TI Endogenous ligands for the **erbB-2** receptor protein that induce cellular responses and their diagnostic and therapeutic uses
IN Lippman, Marc E.; Lupu, Ruth
PA Georgetown University, USA
SO U.S., 61 pp. Cont.-in-part of U.S. Ser. No. 875,788,abandoned.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 5

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5578482	A	19961126	US 1993-96277	19930726
	US 5869618	A	19990209	US 1995-550815	19951031
PRAI	US 1990-528438	19900525			
	US 1991-640497	19910114			
	US 1992-872114	19920422			
	US 1992-875788	19920429			
	US 1992-917988	19920724			
	US 1993-96277	19930726			

AB Endogenous ligands for c-erbB-2 including a 30 kDa transforming growth factor .alpha.-like glycoprotein are identified and characterized. Anti-ligand mols. capable of recognizing and binding to the erbB-2 ligand, screening assays for such ligands, uses for the erbB-2 ligand, the anti-ligand mols., and assays for screening them are also described. A method for inhibiting the growth of human adenocarcinoma cells that overexpress the erbB-2 oncogene by administering the ligand as an inhibitor of cell proliferation is described. A no. of ligands for the receptor were found. The most important was the 30 kDa transforming growth factor .alpha.-like glycoprotein secreted by the estrogen receptor-neg. cell line MDA-MB-231 and reactive polyclonal antibodies to transforming growth factor .alpha.. This is a heparin-binding growth factor capable of strongly binding p185c-erbB2 and inducing its autophosphorylation. It is internalized by the receptor and stimulates the proliferation of c-erbB-2-overexpressing cells at low concns. and inhibits proliferation at high concns. and concomitantly induces differentiation of such cells.

L29 ANSWER 21 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1996:483876 HCAPLUS
DN 125:151113
TI Biosynthetic binding proteins for immunotargeting
IN Huston, James S.; Houston, L. L.; Ring, David B.; Oppermann, Hermann
PA Chiron Corporation, USA; Creative Biomolecules, Inc.
SO U.S., 30 pp. Cont.-in-part of U.S. Ser. No. 831,967,abandoned.
CODEN: USXXAM

DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5534254	A	19960709	US 1993-133804	19931007
	US 5877305	A	19990302	US 1994-356786	19941212
	US 5753204	A	19980519	US 1995-461838	19950605
	US 5837846	A	19981117	US 1995-461386	19950605
PRAI	US 1992-831967	19920206			
	US 1993-133804	19931007			

AB Disclosed is a formulation for targeting an epitope on an antigen expressed in a mammal. The formulation comprises a pharmaceutically acceptable carrier together with a dimeric biosynthetic construct for binding at least one preselected antigen. The biosynthetic construct contains two polypeptide chains, each of which define single-chain Fv (sFv) binding proteins and have C-terminal tails that facilitate the crosslinking of two sFv polypeptides. The resulting dimeric constructs have a conformation permitting binding of a said preselected antigen by the binding site of each said polypeptide chain when administered to said mammal. The formulation has particular utility in in vivo imaging and drug targeting expts.

L29 ANSWER 22 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:267698 BIOSIS
DN PREV199698823827
TI Target cell-specific DNA transfer mediated by a **chimeric** multidomain protein: Novel non-viral gene delivery system.
AU Fominaya, Jesus; Wels, Winfried (1)
CS (1) Inst. Experimental Cancer Res., Tumor Biol. Cent., Breisacher Strasse 117, D-79106 Freiburg Germany
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 18, pp. 10560-10568.
ISSN: 0021-9258.
DT Article
LA English
AB Based on the multidomain structure of the bacterial Pseudomonas exotoxin A, a recombinant fusion protein was constructed which serves as a target cell-specific carrier for the transfer of DNA via **receptor**-mediated endocytosis. The protein consists of three functional domains: (1) an **ErbB-2**-specific single chain **antibody** confers target cell specificity, (2) the exotoxin A translocation domain facilitates endosome escape, and (3) a DNA binding domain derived from the yeast GAL4 protein enables sequence-specific high affinity binding to DNA.
Carrier protein purified from bacterial lysates displayed both **ErbB-2**-specific and DNA sequence-specific binding in vitro. Complexes which formed spontaneously by the interaction of the fusion protein with a luciferase reporter gene construct carrying a GAL4-specific recognition sequence, after condensation of the DNA and compensation of excess negative charge with poly-L-lysine were able to transfect **ErbB-2**-expressing cells in vitro in a cell-specific manner. Transient expression of the luciferase gene driven by the SV40 early promoter was observed and correlates with the amount of carrier protein in the complex. Truncated forms of the carrier protein lacking either the cell recognition domain or the translocation domain

failed to facilitate efficient DNA transfer.

L29 ANSWER 23 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1997:33369 BIOSIS
DN PREV199799339772
TI Targeting of stealth liposomes to **erbB-2** (Her/2) **receptor**: In vitro and in vivo studies.
AU Goren, D.; Horowitz, A. T.; Zalipsky, S.; Woodle, M. C.; Yarden, Y.; Gabizon, A. (1)
CS (1) Dep. Oncol., Hadassah Med. Centre, Jerusalem 91120 Israel
SO British Journal of Cancer, (1996) Vol. 74, No. 11, pp. 1749-1756. ISSN: 0007-0920.
DT Article
LA English
AB Long-circulating (stealth) liposomes coated with polyethylene glycol (PEG), which show reduced uptake by the reticuloendothelial system (RES) and enhanced accumulation in tumours, were used for conjugation to monoclonal **antibodies** (MAbs) as a drug-targeting device. A MAb (N-12A5) directed against **erbB-2** oncoprotein, a functional surface antigen, was used. Amplification and overexpression of the **erbB-2** gene product, being unique to malignancy, confer onto this **antibody**-mediated therapy high tumour specificity. In vitro binding of (3H)cholesteryl ether ((3H)Chol ether) labelled anti-**erbB-2** conjugated liposomes to N-87 cells (**erbB-2**-positive human gastric carcinoma) was compared with the binding of non-targeted liposomes and indicated a 16-fold increase in binding for the targeted liposomes. No difference in binding to OV1063 cells (**erbB-2**-negative human ovary carcinoma) was observed. These results indicate highly selective binding of **antibody**-targeted liposomes to **erbB-2** -overexpressing cells. Despite increased cell binding, doxorubicin (DOX) loaded in anti-**erbB-2**-conjugated liposomes did not cause increased in vitro cytotoxicity against N-87 cells, suggesting lack of liposome **internalisation**. In vivo, the critical factor needed to decrease the non-specific RES uptake and prolong the circulation time of **antibody**-conjugated liposomes is a low protein to phospholipid ratio (lt 60 mu-g mu-mol-1). Using these optimised liposome preparations loaded with DOX and by monitoring the drug levels and the (3H)Chol ether label, biodistribution studies in nude mice bearing subcutaneous implants of N-87 tumours were carried out. No significant differences in liver and spleen uptake between **antibody** -conjugated and plain liposomes were observed. Nevertheless, there was no enhancement of tumour liposome levels over plain liposomes. Both liposome preparations considerably enhanced DOX concentration in the tumour compared with free drug administration. Therapeutic experiments with N-87 tumour-bearing nude mice indicated that anti-tumour activity of targeted and non-targeted liposomes was similar, although both preparations had an increased therapeutic efficacy compared with the free drug. These studies suggest that efficacy is dependent on drug delivery to the tumour and that the rate-limiting factor of liposome accumulation in tumours is the liposome extravasation process, irrespective of liposome affinity or targeting to tumour cells.

L29 ANSWER 24 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1996:706713 HCAPLUS
DN 126:46046

TI Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the **complementarity determining regions** in the center of the antibody binding site
AU Schier, Robert; McCall, Adrian; Adams, Gregory P.; Marshall, Keith W.; Merritt, Hanne; Yim, Michael; Crawford, Robert S.; Weiner, Louis M.; Marks, Cara; Marks, James D.
CS Dep. Anesthesia Pharmaceutical Chem., Univ. California, San Francisco, CA, 94110, USA
SO J. Mol. Biol. (1996), 263(4), 551-567
CODEN: JMOBAK; ISSN: 0022-2836
PB Academic
DT Journal
LA English
AB We detd. the extent to which addnl. binding energy could be achieved by diversifying the complementarity detg. regions (CDRs) located in the center of the antibody combining site of C6.5, a human single-chain Fv (scFv) isolated from a non-immune phage library which binds the tumor antigen c-erbB-2. CDR3 of the light (VL) and heavy (VH) chain variable region of C6.5 were sequentially mutated, the mutant scFv displayed on phage, and higher affinity mutants selected on antigen. Mutation of VL CDR3 yielded a scFv (C6ML3-9) with a 16-fold lower Kd (1.0 .times. 10-9 M) than C6.5. Due to its length of 20 amino acids, four VH CDR3 libraries of C6ML3-9 were constructed. The greatest increase in affinity from a single library was ninefold (Kd = 1.1 .times. 10-10 M). Combination of mutations isolated from sep. VH CDR3 libraries yielded addnl. ninefold decreases in Kd, resulting in a scFv with a 1230-fold increase in affinity from wild-type C6.5 (Kd = 1.3 .times. 10-11 M). The increase in affinity, and its abs. value, are comparable to the largest values obsd. for antibody affinity maturation in vivo or in vitro and indicate that mutation of VL and VH CDR3 may be a particularly efficient means to increase antibody affinity. This result, combined with the location of amino acid conservation and substitution, suggests an overall strategy for in vitro antibody affinity maturation. In addn., the affinities and binding kinetics of the single-chain Fv provide reagents with potential tumor targeting abilities not previously available.

L29 ANSWER 25 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 4
AN 1996:267686 BIOSIS
DN PREV199698823815
TI Expression of **chimeric** envelope proteins in helper cell lines and integration into Moloney murine leukemia virus particles.
AU Schnierle, B. S.; Moritz, D.; Jeschke, M.; Groner, B. (1)
CS (1) Inst. Exp. Cancer Res., Tumor Biol. Cent., Breisacher Str. 117, D-79106 Freiburg Germany
SO Gene Therapy, (1996) Vol. 3, No. 4, pp. 334-342.
ISSN: 0969-7128.
DT Article
LA English
AB New retroviral constructs with a grafted specificity of infection could become useful gene delivery vehicles with applications in systemic gene therapy. We have constructed retroviral vectors to target gene transfer to

human tumor cells. **Chimeric** envelope proteins have been expressed to obtain viral particles with a defined specificity of infection. Two tumor cell-specific recognition domains were cloned and fused with the viral envelope gene. A recognition domain specific for **ErbB-2** expressing tumor cells was derived from a monoclonal **antibody** directed against the **ErbB-2 receptor** in the form of a single chain **antibody** domain (scFv-**erbB-2**). The **receptor** binding domain was derived from the heregulin gene (HRG70). This domain provides recognition specificity for ErbB-3 and ErbB-4 **receptor** expressing tumor cells. The recognition domains were inserted at the amino terminal end into the MoMLV envelope gene. Helper cell lines were established which express the recombinant envelope protein genes, the gag and pol genes and packageable retroviral RNA. The analysis of the helper cell line revealed that the recombinant **ErbB-2** scFv-envelope protein was expressed, but not incorporated into viral particles. The scFv-**erbB-2**-envelope protein was not inserted into the cell membrane and the assembly of retroviral particles was not completed. In contrast, the HRG70-envelope protein was expressed on the surface of the helper cells and incorporated into retroviral particles. The HRG70-envelope protein, however, did not alter the host range of infection. Only cells expressing the ecotropic viral **receptor** could be infected.

L29 ANSWER 26 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:481666 BIOSIS
DN PREV199699196922
TI ErbB **receptor** activation, cell morphology changes, and apoptosis induced by anti-Her2 monoclonal **antibodies**.
AU Kita, Yoshiko (1); Tseng, Julia; Horan, Thomas; Wen, Jie; Philo, John; Chang, David; Ratzkin, Barry; Pacifici, Robert; Brankow, David; Hu, Sylvia; Luo, Yi; Wen, Duanzhi; Arakawa, Tsutomu; Nicolson, Margery
CS (1) Dep. Immunol., Amgen Inc., Amgen Cent., Thousand Oaks, CA 91320 USA
SO Biochemical and Biophysical Research Communications, (1996) Vol. 226, No. 1, pp. 59-69.
ISSN: 0006-291X.
DT Article
LA English
AB A panel of mAbs were generated against the purified soluble form of **erbB2**/Her2 **receptor**, corresponding to the extracellular region of the **receptor**, and examined for their ability to mimic the **receptor** ligand. Some of the mAbs strongly induced tyrosine phosphorylation of 180-185 kDa proteins, including not only Her2 but also Her3 and Her4 **receptors**, when they were expressed on the surface of breast cancer cells. These mAbs do not cross-react with Her3 or Her4 as demonstrated by competition study. **Receptor** phosphorylation was also observed with the cell lines transfected with Her2 or a **chimeric receptor** consisting of the extracellular domain of Her2 and the transmembrane and cytoplasmic domains of epidermal growth factor **receptor**. Selected mAbs were tested for their ability to change cell morphology, and one specific mAb, mAb74, induced cell morphology changes and apoptosis.

L29 ANSWER 27 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS

AN 1995:249906 BIOSIS
DN PREV199598264206
TI Roles for a cytoplasmic tyrosine and tyrosine kinase activity in the interactions of Neu **receptors** with coated pits.
AU Gilboa, Lilach; Ben-Levy, Rachel; Yarden, Yosef; Henis, Yoav I. (1)
CS (1) Dep. Biochem., George S. Wise Fac. Life Sci., Tel Aviv Univ., Tel Aviv
69978 Israel
SO Journal of Biological Chemistry, (1995) Vol. 270, No. 13, pp. 7061-7067. ISSN: 0021-9258.
DT Article
LA English
AB The neu proto-oncogene product, p185-neu (HER2, c-**ErbB-2**), encodes a cell-surface tyrosine kinase **receptor** with high oncogenic potential, which correlates with increased tyrosine kinase activity and a rapid **receptor internalization** rate. To investigate the interactions and signal(s) leading to the endocytosis of Neu **receptors**, we employed lateral mobility and **internalization** studies. Fluorescence photobleaching recovery measurements revealed that activation of Neu **receptors** (induced by mutation or by agonistic **antibodies**) markedly reduced their mobile fractions. To elucidate the signals involved, other mutants, all carrying a constitutively dimerizing oncogenic mutation, were analyzed. A kinase-negative mutant and a mutant lacking all cytoplasmic tyrosine phosphorylation consensus sequences exhibited high mobile fractions, similar to nonactivated Neu. Retention of a single tyrosine autophosphorylation site (Tyr-1253) out of the five known such sites was sufficient to immobilize a large fraction of the **receptor**. For all mutants, **internalization** correlated with **receptor** immobilization and was blocked by treatments that interfere with coated pit structure, indicating that the immobilization is due to interactions with coated pits. This was supported by the coimmunoprecipitation of alpha-adaptin only with the constitutively activated Neu mutants. We conclude that activated Neu **receptors** become stably associated with coated pits via plasma membrane adaptor complexes (AP-2). Efficient Neu **receptor** endocytosis requires activation, a functional kinase domain, and at least one tyrosine autophosphorylation site.

L29 ANSWER 28 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1995:657022 HCAPLUS
DN 123:53877
TI Construction of a **chimeric antibody** with the therapeutic potential for cancers which overexpress c-erbB-2
AU Liu, Hsiao-Lai C.; Parkes, Debbie L.; Langton, Beatric C.; Xuan, Jian-Ai; Longhi, Michael; Elliger, Susan S.; Chao, Lorrine A.; McGrogan, Michael P.; Brandis, John W.; Shawver, Laura K.
CS Dep. Cell and Molecular Biology, Berlex Biosciences, Richmond, CA, USA
SO Biochem. Biophys. Res. Commun. (1995), 211(3), 792-803
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English
AB We describe the chimerization of a monoclonal antibody directed against the c-erbB-2 protein using a novel PCR method for cloning Ig variable region genes. We also describe the characterization of the chimera and show its potential use for treating cancers which overexpress the c-erbB-2 protein. The genomic DNA fragments of heavy and light chain variable

genes were cloned by PCR using uniquely designed primers which allowed for isolation of genes contg. functional promoters, signal and coding sequences. The chimeric genes were then constructed by linking variable regions of murine genes to human const. .gamma.1 and .kappa. genes. Expression of the chimeric Ig genes resulted in prodn. of properly assembled chimeric antibody with improved biol. properties.

L29 ANSWER 29 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 5
AN 1995:496584 BIOSIS
DN PREV199598520134
TI A spacer region between the single chain **antibody**-and the CD3 zeta-chain domain of **chimeric** T cell **receptor** components is required for efficient ligand binding and signaling activity.
AU Moritz, D.; Groner, B. (1)
CS (1) Institute of Experimental Cancer Research, Tumor Biology Center, Breisacher Strasse 117, D-79106 Freiburg Germany
SO Gene Therapy, (1995) Vol. 2, No. 8, pp. 539-546.
ISSN: 0969-7128.
DT Article
LA English
AB The elimination of tumor cells by cytotoxic T lymphocytes (CTLs) could become the basis of a biological cancer therapy. The recognition specificity of cytotoxic T lymphocytes (CTLs) can be genetically modified by stable introduction of **chimeric** T cell **receptor** genes and thus be directed towards tumor cells. We designed a recombinant T cell **receptor** (TCR) component consisting of a single chain Fv derivative of a monoclonal **antibody** (scFv) serving as the extracellular antigen-binding domain and the zeta-chain of the TCFR/CD3 complex serving as a signal transducing domain. Three **chimeric** **receptor** constructs differing in their molecular structure were derived and their functions in transduced T cells compared. A construct in which the scFv domain, specific for the **ErbB-2** **receptor**, was fused directly to the zeta-chain, and two constructs containing different hinge regions between the functional domains, were made. The hinge regions serve as spacers which increase the distance of the scFv moiety from the plasma membrane. Only the two scFv-zeta **chimeras** containing a hinge region showed **ErbB-2** binding activity, when expressed in T cells. The scFv-zeta construct which lacks a spacer segment did not. Consistently, only the spacer-containing **chimeras** transduced T cell **receptor** signals following **ErbB-2** mediated crosslinking. An increase in intracellular Ca-2+ concentrations and cytokine secretion was observed. **ErbB-2** expressing tumor cells were efficiently lysed by CTLs which expressed the spacer-containing scFv-zeta **chimeras**. Our results will help to optimize the design of recombinant T cell **receptor** components useful in the grafting of a specificity of recognition on to cytotoxic T cells and possibly the gene therapy of cancer.

L29 ANSWER 30 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 6
AN 1995:395765 BIOSIS
DN PREV199598410065
TI Biotechnological and gene therapeutic strategies in cancer treatment.

AU Wels, Winfried; Moritz, Dirk; Schmidt, Mathias; Jeschke, Margit; Hynes, Nancy E.; Groner, Bernd (1)
CS (1) Inst. Experimental Cancer Res., Tumor Biol. Cent., Breisacher Strasse 117, D-79106 Freiburg Germany
SO Gene (Amsterdam), (1995) Vol. 159, No. 1, pp. 73-80.
ISSN: 0378-1119.
DT General Review
LA English
AB New anti-cancer agents are being developed which incorporate cancer-cell-specific recognition functions and are thus able to distinguish between normal and tumor cells. Recognition is dependent on the enhanced expression of antigenic determinants on the surface of tumor cells. The **ErbB-2 receptor** (ErbB-2R) is overproduced in a high percentage of adenocarcinomas arising in the breast, ovary, lung and stomach, when compared to normal cells. The tumor-enriched expression and extracellular accessibility make this **receptor** a suitable target for directed tumor therapy. A gene expressing the single-chain **antibody** molecule (scFv), specific for the extracellular domain of the ErbB-2R, was constructed by joining cDNAs encoding the light- and heavy-chain variable domains of the monoclonal **antibody** (mAb) FRP5. This scFv-encoding gene has been used as a targeting domain for two effectors: (i) A recombinant immunotoxin-encoding gene was constructed by adding sequences encoding a modified Pseudomonas aeroginosa exotoxin A (ETA) to the scFv encoding DNA.

(ii) Cytotoxic T-lymphocytes (CTL) with specificity for ErbB-2R-producing tumor cells were generated by retroviral transfer of a **chimeric** gene which encodes the scFv(FRP5), a hinge region and the zeta-chain of the T-cell **receptor** (TCR) complex. The bacterially produced recombinant immunotoxin scFv(FRP5)-ETA binds specifically to the ErbB-2R and displays both in vitro and in vivo cytotoxic effects selective for tumor cells producing high levels of the ErbB-2R. Target cells expressing the ErbB-2R gene were lysed in vitro with high specificity by the scFv::hinge::zeta-expressing T-cells. The growth of ErbB-2R transformed cells in athymic nude mice was retarded by adoptively transferred scFv::hinge::zeta-expressing CTL. Our results suggest that cytotoxic effector functions targeted to tumor cells via tumor selective-binding domains may become useful therapeutic reagents. The selective tumor cell lysis by CTL grafted in vitro with a novel, MHC-independent recognition specificity could become a gene therapy approach to cancer treatment.

L29 ANSWER 31 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1995:459604 HCAPLUS
DN 122:232666
TI Manufacture of disulfide-stabilized polypeptide fragments retaining ligand-binding specificity by expression of the cloned gene
IN Pastan, Ira H.; Lee, Byungkook; Jung, Sun-Hee; Brinkmann, Ulrich
PA Government of the United States of America, USA
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9429350	A2	19941222	WO 1994-US6687	19940614
	WO 9429350	A3	19950202		

W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

US 5747654	A	19980505	US 1993-77252	19930614
CA 2164984	AA	19941222	CA 1994-2164984	19940614
AU 9472464	A1	19950103	AU 1994-72464	19940614
AU 682705	B2	19971016		
EP 703926	A1	19960403	EP 1994-921956	19940614
EP 703926	B1	19980819		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE

JP 09502862	T2	19970325	JP 1994-502172	19940614
AT 169932	E	19980915	AT 1994-921956	19940614
ES 2124419	T3	19990201	ES 1994-921956	19940614

PRAI US 1993-77252 19930614
WO 1994-US6687 19940614

AB Disulfide-bonded peptides with binding ability and specificity for another peptide, such as the variable region of an antibody mol. are manufd. by expression of the cloned gene. The method is particularly intended for the manuf. of Fv antibody fragments stabilized by a disulfide bond connecting the VH and VL region of the Fv fragment. Preferred sites for introduction of cysteines into the Fv fragment are defined by modeling of the peptides. The .alpha. and .beta. chains of T cells receptors may be similarly stabilized. A series of disulfide-bonded analogs of the variable regions of a monoclonal antibody against the tumor-specific B3 antigen were modelled and structural stability and conformation assessed. One of the most stable of these was used to construct an immunotoxin of a fusion protein of the antibody and Pseudomonas exotoxin. Two plasmids, one for a fusion protein of one variable and the exotoxin, and the second for the second variable region were used in sep. Escherichia coli hosts to manuf. the subunits. The proteins accumulated as inclusion bodies and were solubilized and renatured by std. methods using a redox shuffling mechanism and aggregation inhibitors. After renaturation was complete, a final oxidn. using excess oxidized glutathione was used to obtain the immunotoxin in high yields. The immunotoxin was efficient against B3-presenting tumors with an IC50 of 0.25-0.3 ng/mL against breast (MCF7) and epidermoid (A431) cancer cell lines. The protein was less effective against LNCaP and HTB103 cells (with lower levels of B3 antigens) and ineffective against a B3-neg. leukemia (HUT-102).

L29 ANSWER 32 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1995:412729 HCAPLUS
DN 122:151369
TI Modified glycosidation of fusion proteins of anti-tumor **antibodies** and prodrug activating enzymes and the use of the proteins in the targetted treatment of tumors
IN Bosslet, Klaus; Czech, Joerg; Hoffmann, Dieter
PA Behringwerke AG, Germany
SO Eur. Pat. Appl., 28 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 623352	A2	19941109	EP 1994-106394	19940425

EP 623352 A3 19950222
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE
DE 4314556 A1 19941110 DE 1993-4314556 19930504
AU 9461829 A1 19941110 AU 1994-61829 19940502
AU 684750 B2 19980108
CA 2122745 AA 19941105 CA 1994-2122745 19940503
JP 06319554 A2 19941122 JP 1994-117524 19940506
PRAI DE 1993-4314556 19930504
AB Bifunctional antibody-enzyme conjugates with a modified glycosidation patterns are described for use in the treatment of tumors. The antibody component of the conjugate specifically binds a tumor-specific antigen and the enzyme moiety activates a prodrug. The carbohydrate component includes at least one exposed carbohydrate residue selected from the group: mannose, galactose, N-acetylglucosamine, N-acetyllactose, glucose and fucose and the exposed group is generated by enzymic removal of terminal sialic acid or mannose groups with optional enzymic addn. of the new terminal sugar. Glycosidation contributes to increased relative concn. of the glycoproteins at the site of the tumor, and speeds clearance of the protein from the general circulation and non-tumor sites. The proteins are manufd. with a mammalian glycosidation pattern by expression of the cloned gene in a transgenic animal cell line or animal. Clearance studies carried out in CD-1 nude mice on glycosidated and non-glycosidated fusion proteins of a human .beta.-glucuronidase and a human antibody to a tumor antigen are presented.

L29 ANSWER 33 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 7
AN 1994:303768 BIOSIS
DN PREV199497316768
TI Cytotoxic T lymphocytes with a grafted recognition specificity for **ERBB2**-expressing tumor cells.
AU Moritz, Dirk; Wells, Winfried; Mattern, Juergen; Groner, Bernd (1)
CS (1) Friedrich Meischer-Inst., Schwarzwaldallee 215, P.O. Box 2543, CH-4002 Switzerland
SO Proceedings of the National Academy of Sciences of the United States of America, (1994) Vol. 91, No. 10, pp. 4318-4322.
ISSN: 0027-8424.
DT Article
LA English
AB Experimental approaches which exploit the targeted cytolytic activity of lymphocytes are being developed for cancer therapy. We generated cytotoxic T lymphocytes (CTLs) with specificity for **ERBB2 receptor** -expressing tumor cells. A binding function was conferred directly on the zeta chain of the T-cell **receptor** (TCR) complex to circumvent major histocompatibility complex-restricted antigen recognition through the alpha and beta chains of the TCR. A **chimeric** gene was constructed which encoded a single-chain Fv **antibody** (scFv, consisting of the joined heavy- and light-chain variable domains of a monoclonal **antibody** against the extracellular domain of the **ERBB2 receptor**), a hinge region as a spacer, and the zeta chain of the TCR. This gene was introduced into CTLs by retroviral gene transfer. The signaling potential of the scFv/hinge/zeta **receptors** was demonstrated by secretion of interferon gamma upon

coincubation with **ERBB2**-expressing cells. Target cells expressing the **ERBB2** gene were lysed in vitro with high specificity by the scFv/hinge/zeta-expressing T cells. The growth of **ERBB2**-transformed cells in athymic nude mice was retarded by adoptively transferred scFv/hinge/zeta-expressing CTLs. Transduced CTLs labeled with a fluorescent dye were specifically detected in tumor sections. Our results suggest that tumor cell lysis by CTLs grafted in vitro with a major histocompatibility complex-independent recognition could become a gene-therapy approach to cancer treatment.

L29 ANSWER 34 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1994:361768 BIOSIS
DN PREV199497374768
TI P-185-c-**erbB-2** signaling enhances cisplatin-induced cytotoxicity in human breast carcinoma cells: Association between an oncogenic **receptor** tyrosine kinase and drug-induced DNA repair.
AU Arteaga, Carlos L. (1); Winnier, Angela R.; Poirier, Miriam C.; Lopez-Larraza, Daniel M.; Shawver, Laura K.; Hurd, Stephen D.; Stewart, Stanford J.
CS (1) Dep. Med./Oncol, Vanderbilt Univ., 22nd Ave. South, 1956 TVC, Nashville, TN 37232-5536 USA
SO Cancer Research, (1994) Vol. 54, No. 14, pp. 3758-3765. ISSN: 0008-5472.
DT Article
LA English
AB The c-**erbB-2** (HER-2/neu) protooncogene encodes an Al, 185,000 transmembrane glycoprotein with intrinsic tyrosine kinase activity. Agonistic **antibodies** against p185-c-**erbB-2** enhance the cytotoxic effect of the DNA alkylator, cisplatin, against c-**erbB-2**-overexpressing human carcinoma cells (Hancock et al., Cancer Res., 51: 4575- 4580, 199 1). We have studied the possible association between **receptor** signal transduction and cisplatin-mediated cytotoxicity utilizing the SKBR-3 human breast cancer cell line and the anti-p185 TAb 250 IgG1. TAb 250 induced tyrosine phosphorylation of p185 and the **receptor** substrate phospholipase C-gamma-1, as well as rapid association of these molecules in vivo. Simultaneously with phosphorylation, phospholipase C-gamma-1 catalytic activity measured in a (3H)phosphatidylinositol-4,5-bisphosphate hydrolysis assay was increased 61 +- 12% above control. Preincubation of SKBR-3 cells with the tyrosine kinase inhibitor tyrphostin 50864-2 abrogated the enhancement of drug-mediated cell kill induced by TAb 250. The supraadditive drug/**antibody** effect was not seen in SKBR-3 cells with TAb 263, an anti-p185 IgG1 that does not induce **receptor** signaling or with TAb 250 in MDA-468 breast cancer cells which do not overexpress c-**erbB-2**. In addition, transforming growth factor-alpha increased cisplatin-induced cytotoxicity against NIH 3T3 cells overexpressing an epidermal growth factor **receptor/c-erbB-2 chimera**. Cellular uptake or efflux of (195mPt)cisplatin by SKBR-3 cells was not altered by TAb 250. Finally, simultaneous treatment of SKBR-3 cells with TAb 250 and cisplatin increased cisplatin/DNA intrastrand adduct formation and delayed the rate of adduct decay. Taken together these data support a direct association between p185-c-**erbB-2** signal transduction and inhibition of cisplatin-induced DNA repair.

L29 ANSWER 35 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS

AN 1994:180231 BIOSIS
DN PREV199497193231
TI Ligand-like effects induced by anti-c-**erbB-2** **antibodies** do not correlate with and are not required for growth inhibition of human carcinoma cells.
AU Shawver, Laura K.; Mann, Elaina; Elliger, Susan S.; Dugger, Teresa C.; Arteaga, Carlos L. (1)
CS (1) Dep. Med./Oncol., Vanderbilt Univ., 22nd Ave. South, 1956 TVC, Nashville, TN 37232-5536 USA
SO Cancer Research, (1994) Vol. 54, No. 5, pp. 1367-1373. ISSN: 0008-5472.
DT Article
LA English
AB The c-**erbB-2** gene encodes a M-r 185,000 tyrosine kinase **receptor** (p185) with extensive homology to the epidermal growth factor **receptor**. We have conducted mechanistic studies with several anti-p185 monoclonal **antibodies** (TAb 250, -255, -257, -260, and -263) directed against the extracellular domain of p185 utilizing the SKBR-3, BT474, and SKOV-3 cancer cell lines. Several of these **antibodies** exhibited ligand-mimicking properties: they induced tyrosine phosphorylation of p185; increased the catalytic activity of the **receptor** substrate phospholipase C-gamma-1; exhibited time- and pH-dependent **internalization**; induced **receptor** down-regulation; and increased the turnover of the p185 protein DELTA-3-fold. However, there was not a universal correlation between the **antibody**-mediated ligand-like effects and growth inhibition. TAb 250 inhibited BT-474 cells but did not alter p185 phosphotyrosine content or increase **receptor** turnover in these cells. TAb 260 increased p185 protein turnover but did not affect proliferation of the SKOV-3 cell line. Furthermore, blockade of TAb 250-induced **receptor** phosphorylation with the tyrosine kinase inhibitor tyrphostin 50864-2 did not abrogate TAb 250-mediated growth inhibition of SKBR-3 cells. These data suggest that ligand-like effects mediated by p185 **antibodies** are not critical for the growth inhibition of c-**erbB-2** -overexpressing carcinoma cells.

L29 ANSWER 36 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1994:439147 BIOSIS
DN PREV199497452147
TI Targeted toxins as anticancer agents.
AU Siegall, Clay B.
CS Dep. Mol. Immunol., Bristol-Meyers Squibb, Pharm. Res. Inst., 3005 First Ave., Seattle, WA 98121 USA
SO Cancer (Philadelphia), (1994) Vol. 74, No. 3 SUPPL., pp. 1006-1012. ISSN: 0008-543X.
DT Article
LA English
AB Transformed cells, such as those found in breast cancer, often overexpress a variety of cell surface **receptors** and antigens. **Antibodies** or growth factors that specifically recognize these membrane-bound structures can be linked with protein toxins, resulting in 4-cell-specific cytotoxic reagents. Many of these cytotoxic molecules have been produced and are referred to as oncotoxins, mitotoxins, or immunotoxins, depending on the components of the **chimeric**

molecule. These bifunctional reagents are constructed as either chemical conjugates or fusion proteins between a ligand/**antibody** and a toxin. This report focuses on the use of cytotoxic proteins targeted to epidermal growth factor **receptors**, fibroblast growth factor **receptors**, **erbB-2**/HER-2, and tumor-associated carbohydrate antigens. Using immunotoxin therapy, total regression of established tumors in animal xenograft models have been demonstrated. These results suggest that immunotoxin molecules offer exciting opportunities for the treatment of human cancer.

L29 ANSWER 37 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1994:180009 BIOSIS
DN PREV199497193009
TI Demonstration by two-color flow cytometry that tyrosine kinase activity is
required for down-modulation of the oncogenic neu **receptor**.
AU Brown, Valerie I. (1); Shah, Neelima; Smith, Robert; Hellman, Maria; Jarett, Leonard; Mikami, Yasunori; Cohen, Erik; Qian, Xiaolan; Greene, Mark I.
CS (1) Cent. Receptor Biol. and Div. Immunol., Dep. Pathol. Lab. Med., John Morgan Build., Room 252, 36th and Hamilton Walk, Univ. Pa., Philadelphia, PA 19104 USA
SO DNA and Cell Biology, (1994) Vol. 13, No. 2, pp. 193-209.
ISSN: 1044-5498.
DT Article
LA English
AB Expression of rat oncogenic neu **receptor**, p185T-neu (a growth factor **receptor** with constitutive tyrosine kinase activity), causes cells to become transformed. Treatment with anti-neu **receptor** monoclonal **antibodies** reverts the transformed phenotype by down-modulation of p185T-neu. Monoclonal **antibody** treatment of cells expressing normal neu **receptor**, p185C-neu (which lacks constitutive tyrosine kinase activity), does not result in down-modulation of p185C-neu. To understand further the role the biochemical activity of p185Tneu plays in transformation and endocytosis, we created a series of mutations in p185T-neu. We found that fibroblasts expressing the tyrosine kinase-defective mutants cannot form foci in culture. colonies in soft agar, or tumors in immunocompromised mice. To follow the **antibody**-induced endocytosis of neu **receptors** expressed in these transfectants, we developed a novel two-color flow cytometric assay and confirmed **receptor** localization by electron microscopy. Cells were treated with mAb7.16.4 over time. After 4 hr of **antibody** treatment, less than 50% of full-length p185T-neu and of mutant T691 remained on the cell surface, whereas internal expression of the neu **receptors** within these cells initially increased and then decreased to the original internal **receptor** level. In contrast, the level of kinase-deficient mutated neu **receptors** remaining on the cell surface initially decreased by 35%, but, after 4 hr of **antibody** treatment, the cell surface expression level returned to approximately the original level. Concurrently, fluctuations in expression levels were seen internally over time as well. These cell lines were also treated with gold-conjugated mAb7.16.4. Using electron microscopy, we consistently found the gold particles within multivesicular
bodies of cell lines expressing full-length or mutated neu **receptor**. These data strongly suggest that the fate of the neu **receptor**, once **internalized**, is directed by its tyrosine

kinase activity. When the kinase activity of the neu **receptor** is disrupted, the **receptor** is **internalized** but recycled to the cell surface, whereas neu **receptors** which have constitutive kinase activity are **internalized** and presumably degraded when engaged with anti-neu **receptor** mAb. Understanding the regulation of **receptor** endocytosis, degradation, and recycling will contribute to the development of novel therapeutic protocols to combat human malignancies, particularly those associated with the overexpression of the human homologue of the neu **receptor**, c-**erbB2**.

L29 ANSWER 38 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1994:549936 HCAPLUS
DN 121:149936
TI Establishment and characterization of mouse-human **chimeric** monoclonal **antibody** to erbB-2 product
AU Ishida, Tadao; Tsujisaki, Masayuki; Hinoda, Yuji; Imai, Kohzoh; Yachi, Akira
CS Dep. Intern. Med., Sapporo Med. Univ., Sapporo, 060, Japan
SO Jpn. J. Cancer Res. (1994), 85(2), 172-8
CODEN: JJCREP; ISSN: 0910-5050
DT Journal
LA English
AB A mouse-human chimeric antibody for erbB-2 product was established by a new procedure using heavy chain loss mouse mutant hybridoma and human Ig expression vector. The E401 hybridoma secreted anti-erbB-2 product monoclonal antibody (MoAb) (IgG1, .kappa.). The gene for the mouse variable regions of heavy chain was amplified and cloned by the polymerase chain reaction technique directly from the E401 hybridoma RNA. The variable region of heavy chain was joined with the expression vector, which contains human .gamma.1 const. gene. The expression vector was transfected into heavy chain loss mutant cells E401-12, which produced only murine Ig light chains. A chimeric monoclonal antibody CH401 retained full binding reactivity to erbB-2 product, compared with murine E401 parental antibody. Furthermore, the chimeric MoAb CH401 was much more efficient in supporting antibody dependent cell-mediated cytotoxicity activity against erbB-2-bearing human adenocarcinoma cells than murine MoAb E401. These suggest that a chimeric monoclonal antibody CH401 may be a potent reagent for therapy of human adenocarcinoma.

L29 ANSWER 39 OF 54 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
DUPLICATE 8
AN 1993-272889 [34] WPIDS
CR 1996-333194 [33]; 1998-311318 [27]; 1999-023541 [02]
DNN N1993-209536 DNC C1993-121786
TI New single chain Fv polypeptide binding to C-**erbB-2** tumour antigen - for imaging or treating breast or ovarian cancer etc..
DC B04 D16 S03
IN HOUSTON, L L; HUSTON, J S; OPPERMANN, H; RING, D B
PA (CHIR) CHIRON CORP; (CREA-N) CREATIVE BIOMOLECULES INC; (CETU) CETUS ONCOLOGY CORP
CYC 21

PI WO 9316185 A2 19930819 (199334)* 87p
RW: BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
W: AU CA JP
AU 9336122 A 19930903 (199401)
EP 625200 A1 19941123 (199445) EN
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
WO 9316185 A3 19930930 (199513)
JP 08500962 W 19960206 (199643) 91p
AU 675929 B 19970227 (199717)
US 5877305 A 19990302 (199916)
ADT WO 9316185 A2 WO 1993-US1055 19930205; AU 9336122 A AU 1993-36122 19930205; EP 625200 A1 EP 1993-904938 19930205, WO 1993-US1055 19930205; JP 08500962 W JP 1993-514197 19930205, WO 1993-US1055 19930205; AU 675929 B AU 1993-36122 19930205; US 5877305 A Cont of US 1992-831967 19920206, US 1994-356786 19941212
FDT AU 9336122 A Based on WO 9316185; EP 625200 A1 Based on WO 9316185; JP 08500962 W Based on WO 9316185; AU 675929 B Previous Publ. AU 9336122, Based on WO 9316185
PRAI US 1992-831967 19920206; US 1994-356786 19941212
AB WO 9316185 A UPAB: 19990113
New single chain Fv polypeptide (I) defines a binding state having the binding properties of an immunoglobulin able to bind c-**erbB-2** (or related) tumour antigens. (I) contains at least 2 polypeptide domains (PPD) connected by a polypeptide linker (between the C terminus of one domain and the N terminus of the other). Each PPD comprises a set of **complementarity determining regions** (**CDR**) providing binding to the antigen, interspersed between a set of framework regions (FR).
Also new are (1) DNA encoding (I) and (2) host cells transfected with such DNA.
Opt. (I) is coupled to a detectable residue or to a toxin.
USE/ADVANTAGE - (I) can be used to image cells carrying the specified antigen when coupled to detectable gp. or, when coupled to a toxin or radioisotope, for treatment of tumours which express this antigen (e.g. breast or ovarian cancers). Compared with complete **antibodies** (or their larger fragments), (I) are less susceptible to proteolysis; reach their target (and are cleaned) more rapidly, and have lower non-specific binding and immunogenicity. Some (I) also cause **internalisation** of the antigen and (I)-toxin fusion proteins have 15-200 times greater tumour cell killing activity than chemically crosslinked toxin/whole **antibody** (or Fab) constructs. (I) can also be used (not claimed) in specific binding assays; affinity purificn. and as biocatalysts. Where (I) contains a second binding site specific for CD3, it may also induce **antibody**-dependent cell cytotoxicity.
Dwg.1/4

L29 ANSWER 40 OF 54 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1993-214162 [26] WPIDS
DNC C1993-095057
TI Recombinant and **chimeric antibodies** to C-**ERBB-2** - used as therapeutic and diagnostic agents for tumours expressing C-**ERBB-2**.

DC B04 D16
IN BRANDIS, J W; LIU, H C; MCBROGAN, M P; PARKES, D L; SHAWVER, L K
PA (BERL-N) BERLEX LAB INC
CYC 19
PI WO 9312220 A1 19930624 (199326)* EN 106p
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU CA JP
AU 9332367 A 19930719 (199344)
ADT WO 9312220 A1 WO 1992-US10437 19921204; AU 9332367 A AU 1993-32367 19921204
FDT AU 9332367 A Based on WO 9312220
PRAI US 1991-808462 19911212
AB WO 9312220 A UPAB: 19931116
The following are claimed (A) a recombinant DNA sequence encoding at least one **CDR** region derived from an **antibody** specific for c-erB-2 protein, (B) a recombinant DNA sequence that encodes an **antibody** light chain variable region specific for c-**erbB-2** protein, (C) a recombinant DNA sequence that encodes an **antibody** heavy chain variable region specific for c-**erbB-2** protein, (D) a recombinant DNA vector that comprises a DNA sequence as in (A), (B) or (C), (E) a recombinant DNA sequence that comprises DNA encoding a **chimeric** c-**erbB-2** specific heavy or light chain peptide, the variable region derived from a first genetic source and a constant region derived from a second and different genetic source, (F) a recombinant DNA vector that comprises a DNA sequence as in (E), (G) a host cell that expresses **chimeric** **antibody** chains specific for c-erB-2, and (H) a host cell that expresses a protein encoded by a sequence as in (A).
USE/ADVANTAGE - The **chimeric** or recombinant **antibody** peptides specific for c-**erbB-2** can be used as therapeutic or diagnostic agents for treating mammary and ovary cancers or other tumours expressing c-**erbB-2**. The peptides can also be used to produce anti-idiotypic **antibodies** which can induce antitumour responses. By using human constant regions in **chimeric antibodies**, these regions may interact more effectively with human effector cells of the immune system and reduce any immunogenic response.
Dwg.0/16

L29 ANSWER 41 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1993:499479 BIOSIS
DN PREV199396123486
TI Mitogenic activation of the Ras guanine nucleotide exchange factor in NIH 3T3 cells involves protein tyrosine phosphorylation.
AU Li, Bao-Qun; Subleski, Marianne; Shalloway, David; Kung, Hsiang-Fu; Kamata, Tohru (1)
CS (1) Natl. Cancer Inst., Frederick Cancer Res. and Dev. Cent., P.O. Box B, Bldg. 560, Room 31-76, Frederick, MD 21702-1201 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (1993) Vol. 90, No. 18, pp. 8504-8508.
ISSN: 0027-8424.
DT Article
LA English
AB We report biochemical evidence that epidermal growth factor and platelet-derived growth factor stimulate the Ras guanine nucleotide exchange factor activity in quiescent NIH 3T3 cells. Moreover, the

exchange activity is constitutively enhanced in NIH 3T3 cells transformed by Src and **ErbB2** oncogenic tyrosine protein kinases (TPKs), whereas transformation by oncogenic Mos and Raf does not alter the activity. GTPase-activating protein activity was not affected under these conditions. Overexpression of pp60-c-Src mutants containing activated and suppressor TPK mutations resulted in stimulation and inhibition of the exchange factor activity, respectively. A TPK inhibitor, genistein, prevented the activation of the exchange factor in epidermal growth factor/platelet-derived growth factor-treated cells and src-transformed cells. Furthermore, the exchange factor activity bound to an antiphosphotyrosine **antibody** immunoaffinity column. These findings suggest that the guanine nucleotide exchange factor, but not GTPase-activating protein, plays a major role in the Ras activation in cell proliferation initiated by growth factor **receptor** TPKs and malignant transformation by oncogenic TPKs and that tyrosine phosphorylation of either the exchange factor or a tightly bound protein(s) may mediate the activation of the exchange factor by these TPKs.

L29 ANSWER 42 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1994:52256 HCAPLUS
DN 120:52256
TI Targeting of T lymphocytes to Neu/HER2-expressing cells using **chimeric** single chain Fv receptors
AU Stancovski, Ilana; Schindler, Daniel G.; Waks, Tova; Yarden, Yosef; Sela, Michael; Eshhar, Zelig
CS Dep. Chem. Immunol., Weizmann Inst. Sci., Rehovot, 76100, Israel
SO J. Immunol. (1993), 151(11), 6577-82
CODEN: JOIMA3; ISSN: 0022-1767
DT Journal
LA English
AB Cell surface mols. essential for the transformed phenotype or growth of malignant cells are attractive targets for anticancer immunotherapy. Antibodies specific to Neu/HER2, a human adenocarcinoma-assocd. growth factor receptor, were demonstrated to have tumor-inhibitory capacity. Yet, the inefficient accessibility of antibodies to solid tumors limits their clin. use. To redirect effector lymphocytes to adenocarcinomas, the authors constructed and functionally expressed in T cells chimeric single chain receptor genes incorporating both the Ag-binding domain of anti-Neu/HER2 antibodies and the .zeta.-signal-transducing subunit of the TCR/CD3 complex or the .gamma.-signal-transducing subunit of the Ig Fc receptor complex. Surface expression of the anti-Neu/HER2 chimeric genes in cytotoxic T cell hybridomas endowed then with specific Neu/HER2 recognition enabling their activation for IL-2 prodn. and lysis of transformed cells overexpressing Neu/HER2. These chimeric genes hold promise for the immunotherapy of cancer.

L29 ANSWER 43 OF 54 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1992-331479 [40] WPIDS
DNC C1992-147358
TI Recombinant double chain immuno toxin(s) - for treating e.g. graft versus host disease, auto-immune disease (such as type I diabetes), and malignancies (such as adult T-cell leukaemia).
DC B04 D16
IN CHANG, C N; QUEEN, C L
PA (PROT-N) PROTEIN DESIGN LABS INC

CYC 35
PI WO 9215327 A1 19920917 (199240)* EN 48p
RW: AT BE CH DE DK ES FR GB GR IT LU MC NL OA SE
W: AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG MN MW NL NO PL RO RU SD SE
AU 9215826 A 19921006 (199301)
ADT WO 9215327 A1 WO 1992-US1784 19920306; AU 9215826 A AU 1992-15826 19920306, WO 1992-US1784 19920306
FDT AU 9215826 A Based on WO 9215327
PRAI US 1991-666287 19910308
AB WO 9215327 A UPAB: 19931115
Pure recombinant immunotoxin (II) comprises an antigen (Ag) binding component (ABC) and a bacterial toxin polypeptide cpd. The ABC comprise 2 polypeptide chains, each comprising CDRs in an Ig framework region, and the toxin is in peptide linkage to one of the chains.

Pref. the ABC is an Fv or Fab, or a **chimeric** ABC. The framework region is a human Ig framework region. The CDRs are from a different Ig than the framework, region. The ABC binds specifically to a cell surface Ag, esp. a neoplastic or T cell, and comprises the variable domains of anti-Tac. The toxin is an enzyme esp. phospholipase C or PE40. The cell surface Ag may be carcinoembryonic Ag, the **erbB-2** receptor, transferrin receptor, Ags recognised by 17-1A Ab, L6 Ab and B6.2 Ab.

USE/ADVANTAGE - The compsn. can be used to kill cells expressing a human IL-2 receptor. The recombinant double chain IT is more stable and has a longer half life than single chain IT. It has higher affinity for the target cell, and using the compsns. it is possible to maintain IT at lower doses, reducing side effects of treatment. The IT compsns. may be used in combination with other **antibodies** (Abs) or ITs, or with chromotherapeutic or immunosuppressive agents. The compsns. can be used for prophylactic and/or therapeutic treatment of T cell mediated disorders, e.g. graft versus host disease, transplant rejection, autoimmune diseases such as Type I diabetes, multiple sclerosis, rhematoid arthritis, systemic lupus erythematosus and myasthenia gravis, and malignancies such as adult T cell leukaemia, Hodgkins disease and other lymphomagn

Dwg.0/7

L29 ANSWER 44 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1993:167456 HCAPLUS
DN 118:167456
TI Method for making humanized antibodies
IN Carter, Paul J.; Presta, Leonard G.
PA Genentech, Inc., USA
SO PCT Int. Appl., 126 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9222653	A1	19921223	WO 1992-US5126	19920615
	W: AU, CA, JP, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
	CA 2103059	AA	19921215	CA 1992-2103059	19920615
	AU 9222509	A1	19930112	AU 1992-22509	19920615

AU 675916 B2 19970227
EP 590058 A1 19940406 EP 1992-914220 19920615
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE
JP 06508267 T2 19940922 JP 1992-501103 19920615
EP 940468 A1 19990908 EP 1999-105252 19920615
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC
PRAI US 1991-715272 19910614
EP 1992-914220 19920615
WO 1992-US5126 19920615
AB A method for making a humanized antibody is claimed. The method comprises detg. a sequence for a consensus human variable and a consensus human framework region (FR) and comparing these sequences to the corresponding sequences in the non-human antibody. The complementary detg. region (CDR) of the non-human antibody is substituted for that of the consensus human variable region. Residues in the non-human antibody FR which are not homologous to those in the corresponding human consensus FR are substituted for the human residues if the residue noncovalently binds antigen directly, if it interacts with a CDR, or if it participates in the VL-VH interface. The method was used to prep. 8 humanized antibodies to p185HER2 from a mouse monoclonal antibody to this proto-oncogene product. These humanized antibodies were designed to explore the importance of several FR residues. The chimeric genes for the humanized heavy and light chains were transiently expressed in 293 cells, and the affinity of the antibodies for sol. p185HER2 and the anti-proliferative effect on p185HER2-overproducing mammary adenocarcinoma cell line SK-BR-3 were detd. One humanized antibody bound the sol. p185HER2 3-fold more tightly than the murine antibody and had comparable anti-proliferative activity. However, there was no simple correlation of binding affinity and anti-proliferative activity since an increased anti-proliferative activity was not always accompanied by an increased affinity.

L29 ANSWER 45 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1993:100363 HCAPLUS
DN 118:100363
TI Humanized **antibodies** to growth factor **receptor** c-**erbB2** for tumor treatment
IN Wels, Winfried Stephan; Hynes, Nancy E.; Harwerth, Ina Maria; Groner, Bernd; Hardman, Norman; Zwickl, Markus
PA Ciba-Geigy A.-G., Switz.
SO Eur. Pat. Appl., 67 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 502812	A1	19920909	EP 1992-810056	19920127
EP 502812	B1	19960814		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, PT, SE				
AU 9210421	A1	19920813	AU 1992-10421	19920123
AU 662311	B2	19950831		

	AT 141329	E	19960815	AT 1992-810056	19920127
	ES 2091438	T3	19961101	ES 1992-810056	19920127
	CA 2060544	AA	19920806	CA 1992-2060544	19920203
	JP 05192183	A2	19930803	JP 1992-19908	19920205
	US 5571894	A	19961105	US 1994-235838	19940429
	US 5939531	A	19990817	US 1995-465473	19950605
PRAI	EP 1991-810079	19910205			
	US 1991-731190	19910715			
	US 1992-828832	19920131			
	US 1994-235838	19940429			

OS MARPAT 118:100363

AB Humanized antibodies with a mouse variable domains specific for c-erbB2 and a human const. domains (.alpha., .gamma., .delta., .epsilon. or .mu. heavy chain, .kappa. or .lambda. light chain) fused to an effector mol., e.g. enzyme (alk. phosphatase) or toxin (Pseudomonas exotoxin A), and optionally, a peptide spacer facilitating purifn. are prepd. for treating tumors over-expressing the growth factor receptor c-erbB2. Hybridoma cell line FRP5, FSP16, FWP51 and FSP77 were raised by using c-erbB2 receptor protein derived from SKBR3 human breast tumor cell line as antigen. Poly(A)-contg. RNAs were isolated from FRP5 for mol. cloning of plasmids encoding DNA sequences for the chimeric antibodies. Three plasmids, i.e. pWW616, pWW215-5 and pWW215-51 were prepd. and transformed into Escherichia coli for manuf. of three single-chain recombinant antibodies Fv(FRP5)-phoA, Fv(FRP5)-ETA, and Fv(FWP51)-ETA. Fv(FRP5)-phoA was used for immunoassay of c-erbB2 protein in tumors, and Fv(FRP5)-ETA was tested to delay the onset of tumor formation in nude mice.

L29 ANSWER 46 OF 54 HCAPLUS COPYRIGHT 1999 ACS

AN 1993:204781 HCAPLUS

DN 118:204781

TI Recombinant anti-erbB2 immunotoxins containing Pseudomonas exotoxin

AU Batra, Janendra K.; Kasprzyk, Philip G.; Bird, Robert E.; Pastan, Ira; King, C. Richter

CS Lab. Mol. Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA

SO Proc. Natl. Acad. Sci. U. S. A. (1992), 89(13), 5867-71
CODEN: PNASA6; ISSN: 0027-8424

DT Journal

LA English

AB Immunotoxins were made using five different murine monoclonal antibodies to the human erbB2 gene product and LysPE40, a 40-kDa recombinant form of Pseudomonas exotoxin (PE) lacking its cell-binding domain. All five conjugates were specifically cytotoxic to cancer cell lines overexpressing erbB2 protein. The most active conjugate was e23-LysPE40, generated by chem. crosslinking of anti-erbB2 monoclonal antibody e23 to LysPE40. In addn., a recombinant immunotoxin , e23(Fv)PE40, was constructed that consists of the light-chain variable domain of e23 connected through a peptide linker to its heavy-chain variable domain, which in turn is fused to PE40. The recombinant protein was made in Escherichia coli, purified to near homogeneity, and shown to selectively kill cells expressing the erbB2 protooncogene. To improve the cytotoxic activity of e23(Fv)PE40, PE40 was replaced with a variant, PE38KDEL, in which the carboxyl end of PE is changed from Arg-Glu-Asp-Leu-Lys to Lys-Asp-Glu-Leu and amino acids 365-380 of PE are deleted. The e23(Fv)PE38KDEL protein inhibits the growth of tumors formed by the human gastric cancer cell line N87 in immunodeficient mice.

L29 ANSWER 47 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1993:515053 HCAPLUS
DN 119:115053
TI Construction, bacterial expression and characterization of a bifunctional single-chain **antibody**-phosphatase fusion protein targeted to the human **erbB-2 receptor**
AU Wels, Winfried; Harwerth, Ina Maria; Zwickl, Marcus; Hardman, Norman; Groner, Bernd; Hynes, Nancy E.
CS Friedrich Miescher Inst., Basel, CH-4002, Switz.
SO Bio/Technology (1992), 10(10), 1128-32
CODEN: BTCHDA; ISSN: 0733-222X
DT Journal
LA English
AB A single-chain antigen binding protein (scFv) was constructed to recognize the human erbB-2 receptor. In order to express a bifunctional mol., a bacterial alk. phosphatase gene was fused 3' to the scFv gene. The fusion protein [scFv(FRP5)-PhoA] expressed in Escherichia coli specifically recognized the human erbB-2 protein and competed with monoclonal antibody FRP5 for binding to the receptor. The bound scFv(FRP5)-PhoA protein can be detected directly on tumor cells showing that the protein retains both binding and enzymic activity.

L29 ANSWER 48 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1993:2059 BIOSIS
DN PREV199395002059
TI Surface distribution and **internalization** of **erbB-2** proteins.
AU Lotti, Lavinia Vittoria; Di Lazzaro, Clauda; Zompetta, Claudia; Frati, Luigi; Rosaria Torrisi, Maria (1)
CS (1) Dipartimento di Medicina Sperimentale, Universita di Roma "La Sapienza", Viale Regina Elena 324, 00161 Rome Italy
SO Experimental Cell Research, (1992) Vol. 202, No. 2, pp. 274-280.
ISSN: 0014-4827.
DT Article
LA English
AB We report the localization over the cell surface and the early steps of **antibody**-induced **internalization** of the product of the **erbB-2** proto-oncogene, structurally related to the epidermal growth factor **receptor** (EGFR). We show that erbB-2p185 is mostly excluded from endocytic pits on the cell surface. Incubation at 37 degree C with an anti-**erbB-2**/p185 monoclonal **antibody** induces the rapid entry of the protein into the cell. Similar **internalization** is shown by a **chimeric** molecule EGFR/**erbB-2** in response to EGF. Both the timing and the pathway of **internalization** followed by the erbB-2p185 appear totally similar to those described for the EGFR. At variance with the normal erbB-2p185, two mutant activated **erbB-2** proteins are frequently localized within endocytic pits of the cell surface, indicating that mutations in the transmembrane regions may determine constitutive **internalization** of the protein.

L29 ANSWER 49 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1993:167190 HCAPLUS
DN 118:167190

TI Development of humanized bispecific **antibodies** reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene
AU Shalaby, M. Refaat; Shepard, H. Michael; Presta, Len; Rodrigues, Maria L.;
Beverley, Peter C. L.; Feldmann, Marc; Carter, Paul
CS Dep. Cell Biol., Genentech, Inc., South San Francisco, CA, 94080, USA
SO J. Exp. Med. (1992), 175(1), 217-25
CODEN: JEMEAV; ISSN: 0022-1007
DT Journal
LA English
AB The HER2 protooncogene encodes a 185-kD transmembrane phosphoglycoprotein, human epidermal growth factor receptor 2 (p185HER2), whose amplified expression on the cell surface can lead to malignant transformation. Overexpression of HER2/p185HER2 is strongly correlated with progression of human ovarian and breast carcinomas. Recent studies have shown that human T cells can be targeted with bispecific antibody to react against human tumor cells in vitro. Here, a bispecific F(ab')2 antibody mol. was developed consisting of a humanized arm with a specificity to p185HER2 linked to another arm derived from a murine anti-CD3 monoclonal antibody cloned from the UCHT1 hybridoma. The antigen-binding loops for the anti-CD3 were installed in the context of human variable region framework residues, thus forming a fully humanized BsF(ab')2 fragment. Addnl. variants were produced by replacement of amino acid residues located in light chain complementarity detg. region 2 and heavy chain framework region 3 of the humanized anti-CD3 arm. Flow cytometry anal. showed that the bispecific F(ab')2 mols. can bind specifically to cells overexpressing p185HER2 and to normal human peripheral blood mononuclear cells bearing the CD3 surface marker. In addnl. expts., the presence of bispecific F(ab')2 caused up to fourfold enhancement in the cytotoxic activities of human T cells against tumor cells overexpressing p185HER2 as detd. by a 51Cr release assay. These bispecific mols. have a potential use as therapeutic agents for the treatment of cancer.

L29 ANSWER 50 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 9
AN 1991:526649 BIOSIS
DN BA92:138109
TI REQUIREMENTS FOR THE **INTERNALIZATION** OF A MURINE MONOCLONAL **ANTIBODY** DIRECTED AGAINST THE HER-2-NEU GENE PRODUCT C-ERB-B-2.
AU MAIER L A; XU F J; HESTER S; BOYER C M; MCKENZIE S; BRUSKIN A M; ARGON Y; BAST R C JR
CS BOX 3843, DUKE UNIV. MED. CENT., DURHAM, N.C. 27710, USA.
SO CANCER RES, (1991) 51 (19), 5361-5369.
CODEN: CNREA8. ISSN: 0008-5472.
FS BA; OLD
LA English
AB A murine monoclonal **antibody**, TA1, is directed against an epitope on the extracellular domain of the HER-2/neu (c-**erbB-2**) gene product. Requirements for TA1-induced **internalization** of c-**erbB-2** have been studied using the SKBr3 human breast cancer cell line and several rat fibroblast cell lines that express either wild-type or mutant human c-**erbB-2**. **Internalization** of TA1 was monitored by assaying

protease-resistant uptake of 125I-labeled TA1, by electron microscopy of gold-labeled TA1, and by inhibition of clonogenic growth of cells incubated with TA1 that had been conjugated with blocked ricin. Similar rates of **internalization** of TA1 were observed in SKBr3 and in rat fibroblasts that expressed human c-**erbB-2**. The route of endocytosis was the same as that observed with **antibodies** against other membrane **receptors**. Anti-c-**erbB-2** and anti-transferrin **receptor** cointernalized through clathrin-coated pits, coated vesicles, endosomes, and multivesicular bodies. Products of mutant c-**erbB-2** that lacked a portion of the tyrosine kinase domain or that lacked most of the cytoplasmic domain were endocytosed in the presence of TA1 as promptly as the wild-type c-**erbB-2** product. Slightly more rapid **internalization** of TA1 was observed in rat cells that expressed c-**erbB-2** with a single point mutation in the transmembrane . domain. Taken together, our data suggest that neither the intracytoplasmic domain nor **receptor** phosphorylation is required for **antibody**-mediated endocytosis of c-**erbB-2**.

L29 ANSWER 51 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1991:160949 BIOSIS
DN BA91:86749
TI SCINTIGRAPHIC DETECTION OF OVEREXPRESSED C-ERB-B-2 PROTOONCOGENE PRODUCTS BY A CLASS-SWITCHED MURINE ANTI-C-ERB-B-2 PROTEIN MONOCLONAL **ANTIBODY**.
AU SAGA T; ENDO K; AKIYAMA T; SAKAHARA H; KOIZUMI M; WATANABE Y; NAKAI T; HOSONO M; YAMAMOTO T; ET AL
CS DEP. NUCL. MED., KYOTO UNIV. HOSP., SHOGOIN, SAKYO-KU, KYOTO 606, JPN.
SO CANCER RES, (1991) 51 (3), 990-994.
CODEN: CNREA8. ISSN: 0008-5472.
FS BA; OLD
LA English
AB Class-switched monoclonal **antibody** SV2-61r recognizes the extracellular domain of c-**erbB-2** protooncogene products separate from the epidermal growth factor **receptor**. We studied the potential of SV2-61r for evaluating the amplification of c-**erbB-2** protooncogene on cancer cells, which has been reported to have prognostic value in adenocarcinoma patients. Radiolabeled SV2-61r specifically bound to various adenocarcinoma cells in addition to c-**erbB-2**-transfected NIH-3T3 cells (A4) with the affinity constant of 4.4 .times. 108 M-1. SV2-61r injected i..v. localized well to A4 cells xenografted in nude mice. Tumor uptake and localization index of radioiodinated SV2-61r were lower than those of 111In-labeled SV2-61r, probably due to the **internalization** and dehalogenation of formed antibdy-antigen complexes. Biodistribution and specificity of targeting were assessed by comparison among three cells, A4 lung cancer SBC-3 (c-**erbB-2** weakly positive) and B-lymphoblastoid Manca cells (c-**erbB-2** negative). Tumor:blood ratios, obtained 48 h after injection, were 5.63, 1.45, and 0.68, respectively, indicating the potential of 111In-labeled SV2-61r for evaluating the amplification of c-**erbB-2** protooncogene on cancer cells. Because of its close relationship with carcinogenesis and the uniform expression, c-**erbB-2** protooncogene products seem to be the optimal target of imaging and therapy of adenocarcinoma patients.

L29 ANSWER 52 OF 54 HCAPLUS COPYRIGHT 1999 ACS
AN 1991:406629 HCAPLUS
DN 115:6629
TI Selection of monoclonal **antibodies** which induce **internalization** and phosphorylation of p185HER2 and growth inhibition of cells with HER2/NEU gene amplification
AU Tagliabue, Elda; Centis, Filippo; Campiglio, Manuela; Mastroianni, Antonio; Martignone, Stefania; Pellegrini, Rita; Casalini, Patrizia; Lanzi, Cinzia; Menard, Sylvie; Colnaghi, Maria I.
CS Div. Exp. Oncol. E, Ist. Naz. Studio Cura Tumori, Milan, 20133, Italy
SO Int. J. Cancer (1991), 47(6), 933-7
CODEN: IJCNAW; ISSN: 0020-7136
DT Journal
LA English
AB In order to obtain further information on the biol. role of the HER2/neu oncoprotein monoclonal antibodies (MAbs) were produced against the p185 extracellular domain. To immunize the mice and screen the hybridoma supernatants a lung adenocarcinoma cell line was selected (Calu-3), which demonstrated an overexpression of p185HER2 measured as the reactivity with polyclonal rabbit serum to the 14-amino acid C-terminal-HER2/neu. Two MAbs, MGR2 (IgG1) and MGR3 (IgG2), selected for reactivity on Calu-3 and negativity on A431 live cells, the ref. target cell for EGF receptor expression, immunopptd. an 185 kDa mol. Immunodepletion expts. with the polyclonal antiserum and cross-competition expts. indicated that the 2 reagents recognized 2 different epitopes located on the p185HER2 mol. One of the 2 MAbs, MGR3, was found to internalize, induce p185HER2 phosphorylation, and inhibit tumor cell growth in vitro. Thus, MGR3 is directed against a determinant located in the p185HER2 ligand binding site and may compete with the p185HER2 ligand, but is incapable of inducing a complete mitotic signal.

L29 ANSWER 53 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1991:138617 BIOSIS
DN BA91:75157
TI THE NORMAL **ERBB-2** PRODUCT IS AN ATYPICAL **RECEPTOR**-LIKE TYROSINE KINASE WITH CONSTITUTIVE ACTIVITY IN THE ABSENCE OF LIGAND.
AU LONARDO F; DI MARCO E; KING C R; PIERCE J H; SEGATTO O; AARONSON S A; DI FIORE P P
CS LAB. CELLULAR MOLECULAR BIOL., NCI, NIH, BUILD. 37, ROOM 1D23, BETHESDA, MD. 20892.
SO NEW BIOL, (1990) 2 (11), 992-1003.
CODEN: NEBIE2. ISSN: 1043-4674.
FS BA; OLD
LA English
AB Overexpression of the **erbB-2**/neu gene is frequently detected in human cancers. When overexpressed in NIH 3T3 cells, the normal **erbB-2** product, gp185erbB-2, displays potent transforming ability as well as constitutively elevated levels of tyrosine kinase activity in the absence of exogenously added ligand. To investigate

the basis for its chronic activation we sought evidence of a ligand for gp185erbB-2 either in serum or produced by NIH 3T3 cells in an autocrine manner. We demonstrate that a putative ligand for gp185erbB-2 is not contained in serum. **Chimeric** molecules composed of the extracellular domain of gp185erbB-2 and the intracellular portion of the epidermal growth factor **receptor** (EGFR) did not show any transforming ability or constitutive autophosphorylation when they were expressed in NIH 3T3 cells. However, they were able to transduce a mitogenic signal when triggered by a monoclonal **antibody** directed against the extracellular domain of **erbB-2**. These results provide evidence against the idea that an **erbB-2** ligand is produced by NIH 3T3 cells. Furthermore, we obtained direct evidence of the constitutive enzymative activity of gp185erbB-2 by demonstrating that the **erbB-2** kinase remained active in a **chimeric** configuration with the extracellular domain of the EGFR, in the absence of any detectable ligand for the EGFR. Thus, under conditions of overexpression, the normal gp185erbB-2 is a constitutively active kinase able to transform NIH 3T3 cells in the absence of ligand.

L29 ANSWER 54 OF 54 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1989:181302 BIOSIS
DN BA87:92568
TI A **CHIMERIC** EGF-R-NEU PROTO-ONCOGENE ALLOWS EGF TO REGULATE NEU TYROSINE KINASE AND CELL TRANSFORMATION.
AU LEHVASLAIHO H; LEHTOLA L; SISTONEN L; ALITALO K
CS DEP. VIROL. PATHOL., UNIV. HELSINKI, HAARTMANINKATU 3, SF-00290 HELSINKI, FINL.
SO EMBO (EUR MOL BIOL ORGAN) J, (1989) 8 (1), 159-166.
CODEN: EMJODG. ISSN: 0261-4189.
FS BA; OLD
LA English
AB The neu oncogene, characterized by Weinberg and colleagues, is a transforming gene found in ethylnitrosourea-induced rat neuro/glioblastomas; its human protooncogene homologue has been termed **erbB2** or HER2 because of its close homology with the epidermal growth factor **receptor** (EGF-R) gene (c-erbB1). Expression of the rat neu oncogene is sufficient for transformation of mouse NIH 3T3 fibroblasts in culture and for the development of mammary carcinomas in transgenic mice, but the neu proto-oncogene has not been associated with cell transformation. We constructed a vector for expression of a **chimeric** cDNA and hybrid protein consisting of the EGF-R extracellular, transmembrane and protein kinase C-substrate domains linked to the intracellular tyrosine kinase and carboxyl terminal domain of the rat neu cDNA. Upon transfection with the construct, NIH 3T3 cells gave rise to EGF-R antigen-positive cell clones with varying amounts of specific EGF binding. Immunofluorescence and immunoprecipitation using neu- and EGF-**receptor** specific **antibodies** demonstrated a correctly oriented and positioned **chimeric** EGF-R-neu protein of the expected apparent mol. wt on the surface of these cells. EGF or TGF.alpha. induced tyrosine phosphorylation of the **chimeric receptor** protein, stimulated DNA synthesis of EGF-R-neu expressing cells and led to a transformed cell morphology and growth in soft agar. In contrast, the neur proto-oncogene did not show kinase activity or transforming properties when expressed at similar levels in NIH 3T3 cells.

These results suggest that the neu proto-oncogene possesses mitogenic and transforming properties only in the presence of a ligand which stimulates its tyrosine kinase activity and provides the first model for studies of the function of the neu tyrosine kinase.